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The American Phytopathological Society

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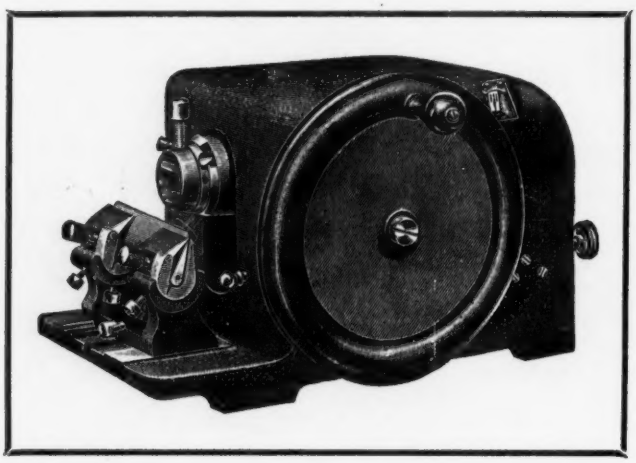

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PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

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PHYTOPATHOLOGY

VOLUME 21 NUMBER 10

OCTOBER, 1931

THE TOXICITY OF WATER-SOLUBLE EXTRACTIVES OF WESTERN YELLOW PINE TO LENZITES SEPIARIA[1]

BERNARD A. ANDERSON

INTRODUCTION

The following study of the toxicity to fungi of water-soluble substances, extracted from western yellow or Pondosa pine, *Pinus ponderosa* Lawson, and of volatile oils driven off during kiln drying, has been undertaken to furnish additional information on the durability of Pondosa pine.

Extraneous components of wood that include tannins, resins, dyes, gums, oils, alkaloids, etc., are chemical products of the tree. These components may be found in some woods and absent in others. They are usually present in the xylem of the tree and can be extracted by suitable solvents. According to Hawley and Wise (4), these substances are not considered an integral part of the cell wall.

At the present time research work is being carried on in the Forest Research Laboratory at the University of Idaho on various factors influencing the decay of wood. This experimental work is part of a program for the determination of the uses to which Inland Empire woods are especially adapted.

It is well known that in nearly all commercial species the durability of timber varies with the amount of heartwood and sapwood (7). Heartwood is generally more durable than sapwood (5). Accurate information covering the relative durability of heartwood and sapwood of various species, however, is comparatively scarce.

Pondosa pine may contain from 2 to 12 inches of sapwood, varying with the maturity of the tree. Service records prove that Pondosa pine heartwood is more durable than the sapwood. Is, then, the difference in durability of heartwood and sapwood due to the presence of sugars, protoplasm, or starches in the sapwood? Is it due to the presence of resin or oils or to the deposition of certain substances in the heartwood? Or, is it due to the presence of water-soluble toxic substances in the heartwood not found in the sapwood? These questions are as yet not answered and an attempt to

[1] Presented as a thesis for the Master's Degree in Forestry. School of Forestry, University of Idaho.

gather these data is here recorded. It is principally with the water-soluble substances that this paper deals.

Rose and Lisse (11) in their work on the chemistry of wood decay in 1917, found that the amount of hot- and cold-water extractives secured from wood varied with the stage of decay. In percentage relationship of extract to wood there was a decrease of cold-water extract from 4.03 per cent in sound Douglas fir heartwood to 1.16 per cent; in hot-water extracts, an increase of from 2.23 per cent to 7.77 per cent. The results of the cold-water extraction probably are not reliable because, as Rose and Lisse explain, "quite evidently the first of these could hardly be reliable because with increasing solubility in cold water there would be an increasing tendency towards loss by leaching out." The results would undoubtedly vary with the type of rot organism present in the wood, since fungi seem to vary in the manner in which they attack the components of wood (5).

A large number of heartwoods that are durable and resistant to decay under actual service conditions have been shown to contain water-soluble extractives in large amounts (3). On the other hand, hardwoods readily attacked by decay are usually deficient in such extractives. The kind of extractive varies with the species. In chestnut, black locust, oak, and red mulberry tannin exists; in Osage orange and black walnut, a soluble coloring matter is found in large quantities; redwood possesses an unknown coloring matter (3); western red cedar also contains a highly toxic brownish extractive (13). From such nondurable woods as birch, maple, or red alder, only indefinite and colorless extractives have been secured (3).

In the toxicity tests carried out by Sowder (13) and Hawley (3), hot-water extracts from both heartwood and sapwood were found more toxic than corresponding cold-water extracts. This probably is due to the increased amount of extractives or to a difference in the nature of the extractives. The above workers (3) (13) also have found that in all cases the sapwood extracts are less toxic than corresponding heartwood extracts.

A very interesting example of the practical application and the importance of a thorough knowledge of water-soluble extractives of wood is the problem offered by "box scald." Box scald is the term applied to a certain type of injury to apples and pears resulting from contact with wooden containers (2). It is a brownish discoloration on the skin of the apple, with surface roughened as if it had been pressed against the board and subjected to abrasion. Tests have disclosed the fact that the injury is due to a water-soluble substance present in the heartwood but absent in the sapwood of Douglas fir. Western hemlock, western yellow pine, and Sitka spruce do not contain the injurious extractive.

The extractives in the tests reported in this paper were secured from air-seasoned and kiln-dried lumber. The kiln-dried stock was dried at a

kiln temperature of not higher than 180° F., which is not a severe dry-bulb temperature for Pondosa pine. It is reasonable to suppose that when kiln-drying lumber and subjecting it to temperatures that run as high as 200° F. (14) certain volatile constituents of the wood are driven off.

It is possible that the high temperatures used in kiln drying affect the durability of the wood. Koehler and Thelen (6) state: "Whether the removal of moisture in itself causes any chemical changes in wood substance is not known, but it is a well-established fact that heat, if severe enough, will cause a decided chemical change in wood with a corresponding reduction in strength." Little, however, is known of the relative durability of air-seasoned and kiln-dried stock.

Under ordinary conditions the decomposition of wood by heat does not progress to any extent until a temperature of 275° C. is reached (4). Up to this point all water is driven off, and possibly extraneous matter is volatilized, but no decided chemical change occurs in the wood. The products driven off at temperatures up to 280° C. to 290° C. are water, a small amount of acetic acid, and a trace of methanol. Active decomposition of wood into primary tar and primary charcoal occurs when the temperature is raised above this point. It is possible, however, that the same chemical changes may result in wood subjected to medium high temperatures for a long period as those changes that occur when the wood is subjected to a high temperature for a short period. The slow darkening of wood in contact with steam pipes (4) may be such a change.

The work of Bateman (1) on the effect of resin on durability leads us to believe that there is a definite relationship between the volatile oil content of resinous wood and durability. A certain relationship between resin, an extraneous component, and durability could be expected, since it is known that certain hydrocarbons, as toluene and benzene, etc., are very toxic to fungi (1). Experiments on the toxicity of terpenes and terpene alcohols by Bateman showed a strong retardation of the test organism, although no actual killing of the organism occurred. Cymene, the main constituent of spruce, turpentine, alpha pinene, the main constituent of gum turpentine from long-leaf pine, and beta pinene, the main constituent of gum turpentine from western yellow pine, showed a respective retardation of 98 per cent, 98 per cent, and 97 per cent.

Pine oil is extremely toxic. In tests (1) to determine the cause of the durability of an exceptionally durable, very resinous, heartwood long-leaf pine tie, it was found upon steam-distilling the tie that it contained ten times as much pine oil as was necessary to prevent the growth of decay organisms. Pine oil, one of the volatile oils found in yellow pine, is not present in "oleoresin." Oleoresin (4) is the viscous substance secreted

by the resin cells of the sapwood when the tree is wounded. Resin, which does contain small amounts of pine oil, is considered as an extraneous substance in the wood proper. Pine oil is probably an oxidation product, formed in the presence of air and water.

METHODS

Only actual service tests will give conclusive proof of the effects of kiln drying on lumber. Satisfactory service tests, however, cannot be carried out in less than 20 or 30 years. Laboratory tests on problems of this kind are merely indicatory of what the results probably will be. They are open to many criticisms, but at least they can be used as a basis and guide for conducting service tests.

Investigators in the past have used two principal methods in carrying on toxicity tests in the laboratory. In the first, the preservative is mixed with sawdust or injected into the wood and the fungus grown on a wood base, and, in the second, the preservative is mixed with some nutrient-agar solution. The nutrient solution varies from a malt to a beef or vegetable extract (9).

The growth of any particular fungus on different media varies and thereby renders noncomparable the results of toxicity tests carried out with the same fungus but on different media. The tendency (9) in late years has been to make all tests on a malt-agar medium, thus giving a uniformity in all tests. Therefore, all cultures in the following work were grown on a malt-agar medium. Hawley, Fleck, and Richards (3), in their investigations on toxic extracts of various woods, and Sowder (13), in his work on cedar extracts, used this type of medium.

Lenzites sepiaria (Fries), the fungus causing a brown cubical rot, was selected for the toxicity tests. Two factors were given special consideration in choosing a fungus for such tests: First, a fungus was wanted that actively attacks Pondosa-pine stock, in service; second, it was desirable to have a fungus that would be fairly sensitive to varying concentrations of preservative. Tests carried out by Schmitz (12) and Richards (10) indicate that *L. sepiaria* would give satisfactory results.

Characteristics of wood used in tests: Four pieces of western yellow or Pondosa pine were selected from which to secure the water-soluble extracts to be tested. These pieces were:

1. Kiln-dried heartwood.
2. Kiln-dried sapwood.
3. Air-seasoned heartwood.
4. Air-seasoned sapwood.

Care was used in selecting the stock to secure pieces of normal Pondosa pine, and all pieces were taken large enough to allow for any varying densities within the wood. Any pieces with pitch pockets, decay, knots, or blemishes of any kind were thrown out.

The number of rings per inch was then determined for each piece, as a large number of rings per inch sometimes indicates a dense wood. Within a given species the denser the wood, the more resistant it is to decay (15). To determine the moisture content and the specific gravity of each board used for each type of wood floor, an inch block was taken from the exact center of each. This position tends to give a truer average for the board.

Standard methods were used to compute the percentage of moisture and the specific gravity of each block (8).

Preparation of water-soluble extracts: The sawdust was ground into wood flour of such fineness that it passed through a 0.5 mm. sieve. Hot- and cold-water extracts were then prepared by weighing out duplicate sets of 300 gm. of each kind of wood flour; 3,000 cc. of distilled water at room temperature was then added to the one sample, and the same amount of boiling, distilled water was added to the duplicate. The cold-water sample was stirred intermittently and allowed to soak for 48 hours. At the end of this time, which was deemed long enough to leach out all water-soluble extracts from such fine particles of wood flour, the extract was drained off and the wood flour washed with 6,000 cc. of cold, distilled wash water, added to the soaking water. The hot-water sample was allowed to soak in a boiling-water bath for 3 hours, and then it was washed with 6,000 cc. of boiling, distilled water and the wash waters added to the soaking water.

The cold- and hot-water extracts were then passed through filter paper. The filtrate was evaporated down to 300 cc. at a temperature never exceeding 70° C. One cc. of filtrate, then, represented the amount of extract secured from 1 gram of wood flour. The extracts, with the exception of the kiln-dried and air-seasoned cold-treated heartwoods, filtered readily in from 2 to 12 hours. The two exceptions filtered slowly, requiring, respectively, 5 and 8 days.

No precipitation occurred in any of the filtrates before they were evaporated down. No distinct discoloration of any of the filtrates was noticeable. Each showed a slightly cloudy appearance, with a faint grayish to yellowish green tinge. As the filtrates were evaporated down to 300 cc., precipitates appeared. The amount and color of the precipitates varied with the type of filtrate.

DISCUSSION OF WATER-SOLUBLE EXTRACTS

The wood flour showed a slight darkening of the original color after having been treated with hot or cold water. Before treating, the kiln-dried heartwood showed a slightly darker light buff color than the air-seasoned heartwood. There was no difference in color between the kiln-dried and air-seasoned sapwood, either before or after treating.

In every instance the extracts from cold-treated wood flour were darker than the extracts from the corresponding hot-treated wood flour. Kiln-dried sapwood extracts were darker than the kiln-dried heartwood extracts and the same was true for the air-seasoned sapwood and heartwood extracts. The precipitates from the hot- and cold-treated kiln-dried heartwood and the hot-treated air-seasoned sapwood were the same color as the solution of the respective extracts. In all of the other extracts the color of the precipitate varied from that of the solution.

The amount of water-soluble material removed from the wood flour by the hot- and cold-water treatments was determined by measuring out 5 cc. of the extract in a watch glass. This sample was evaporated to dryness at a temperature of about 40° C. The residue was then weighed and the original volume (5 cc.) divided by the weight of the residue, since 1 cc. of the extract represents 1 gm. of wood flour, and this result, times 100, gave the percentage of water-soluble substance removed from the wood.

The amount of water-soluble material removed from each of the various extracts was as follows:

	Hot treated	*Cold treated*
Kiln-dried heartwood	0.1165 gm. or 2.33%	0.056 gm. or 1.12%
Kiln-dried sapwood	0.079 " " 1.58 "	0.045 " " 0.9 "
Air-seasoned heartwood	0.180 " " 3.6 "	0.089 " " 1.78 "
Air-seasoned sapwood	0.119 " " 2.38 "	0.063 " " 1.26 "

The hot-water treatment removed more water-soluble material from both the heartwood and sapwood than was removed by the cold-water treatment. Approximately twice as much material was dissolved by the hot water. The heartwood of the kiln-dried stock contained about a third more water-soluble material than the kiln-dried sapwood. The same was true of the air-seasoned heartwood and sapwood. This was to be expected since the densities of the heartwood were considerably greater than those of the sapwoods.

Litmus tests of the different extracts gave a slightly acid reaction for the hot- and cold-treated air-seasoned sapwood. The other extracts gave no reactions, whatever.

TOXICITY TESTS

Methods: The toxic qualities of each of the extracts were tested by the Petri-dish method. (2). A malt-agar medium was used. Concentrations of 10, 25, 50, 75, and 100 per cent of each of the extracts were prepared for the tests. Each of these concentrations was prepared in series of 5, so that the results would represent the averages of a large number of Petri-dish tests. Therefore, the results in this paper are based on a total of 240 Petri-dish cultures.

For the 10 per cent and 25 per cent concentrations a 1.5 per cent malt-agar medium was used. The malt agar consisted of 1,000 cc. of distilled water, 25 gm. of Trömmer's malt extract, and 15 gm. of bacto-agar. The medium used for the 50 and 75 per cent concentrations was a 3 per cent malt-agar solution consisting of 1,000 cc. of distilled water, 25 gm. of Trömmer's malt extract, and 30 gm. of bacto-agar. A 3 per cent malt-agar medium was used for the 50 and 75 per cent concentrations instead of the 1.5 per cent medium, so that the concentration could be gelatinized.

The 10 per cent concentrations consisted of 2 cc. of wood-flour extract, 1 cc. of distilled water, and 17 cc. of 1.5 per cent malt agar. The 25 per cent concentrations consisted of 5 cc. of wood-flour extract and 15 cc. of malt agar; the 50 per cent concentrations of 10 cc. of wood-flour extract and 10 cc. of 3 per cent malt agar; and the 75 per cent concentrations of 15 cc. of wood-flour extracts and 5 cc. of 3 per cent malt agar. The 100 per cent concentrations consisted of 20 cc. of wood-flour extract.

The various concentrations were measured out in test tubes, plugged with a cotton plug, and sterilized at 100° C. for 30 minutes on 3 successive days. Each tube was then shaken thoroughly, so as to secure a thorough and even mixture, and then poured into a Petri dish. Great care was taken

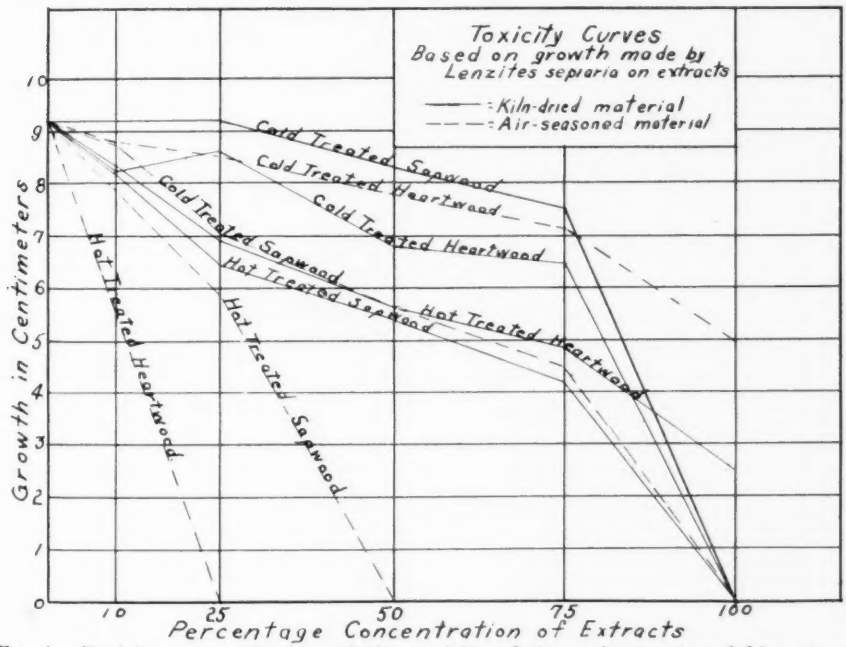

FIG. 1. Toxicity curves showing relative toxicity of the various water-soluble extracts of Pondosa pine based on the growth made by *Lenzites sepiaria* on different concentrations of the extracts.

to keep all dishes free from contamination. All contaminated dishes were discarded.

Each dish was then inoculated with a 0.5 cm. square of a fresh vigorously growing culture of *Lenzites sepiaria*. The culture of the fungus was secured from a transfer in possession of Dr. E. E. Hubert, Forest Products Laboratory, Moscow, Idaho. The culture was originally obtained from a hemlock log at the Forest Products Laboratory, Madison, Wisconsin. The inoculum was grown on a 1.5 per cent malt-agar medium.

To determine the exact effect of the extracts on the growth of the fungus, a series of 5 controls was made for each type of extract. The medium of the control dishes consisted of 20 cc. of 1.5 per cent. malt agar.

The cultures were then placed in a culture case and allowed to grow until the fungus reached the edges of the Petri dish. The culture case was kept at a temperature of about 22° C. Measurements of the diameter growth were taken at various intervals. To secure a correct growth reading of each dish, two measurements were taken at right angles to each other and averaged. The controls required approximately 22 days to reach the edges of the Petri dishes.

GROWTH OF CULTURES

Some of the cultures showed no growth, whatever. After 10 days, to determine whether the fungus had been killed or growth merely inhibited, a transfer was made from those cultures showing no growth and placed on a test-tube slant of 1.5 per cent malt agar. All of the cultures that failed to grow, and are marked with ([a]), in table 1, recovered within 3 weeks when transferred to slants; growth was merely inhibited. Those cultures marked with ([b]) failed to recover; in these cases the fungus was killed by the toxic properties of the extracts. The 100 per cent concentrations of hot-treated air-seasoned sapwood and hot-treated air-seasoned heartwood were the only two extracts that actually killed the fungus.

Photographs were taken of representative cultures of the concentrations of the different types of water-soluble extracts. The plates of each concentration selected for photographing showed a medium condition of growth and were typical for that concentration. Figure 2 shows these cultures, together with the control plate of 1.5 per cent malt agar. They give a comprehensive comparison of the growth of the fungus and the characteristics of the growth, especially as to its luxuriance, diameter growth, subgrowth, and evenness.

The growth of *Lenzites sepiaria* on pure malt agar tends to form a raised ring of aerial growth of an inch radius about the inoculum. The aerial mycelium is white to gray and tinged with yellow, as it ages. The

culture tends to show a bunchy rather than a uniformly even growth of mycelium.

The percentage of retardation of the growth made by the culture was secured by dividing the growth of the culture by that of the growth on the control and subtracting the result from 1 and multiplying by 100.

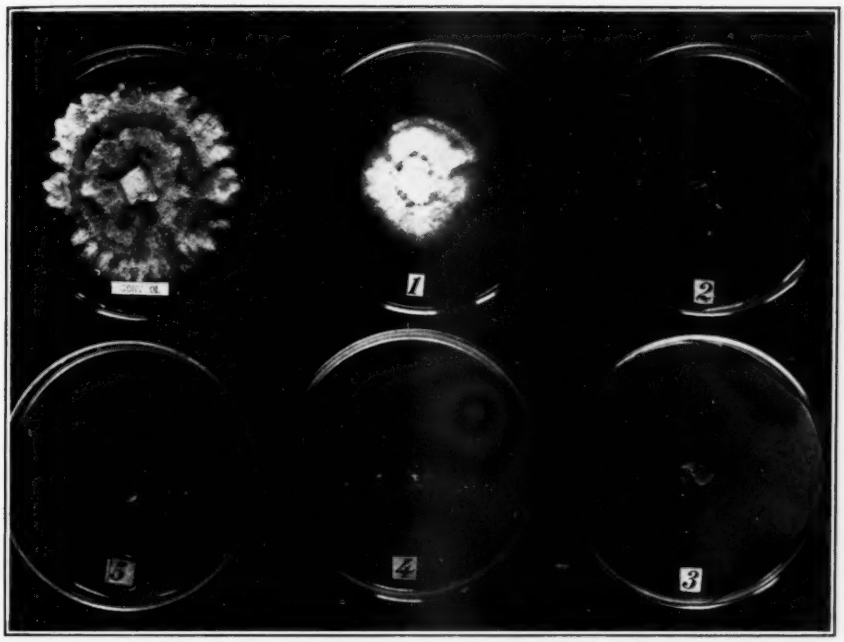

FIG. 2. Photograph showing growth of *Lenzites sepiaria* made on control of 1.5 per cent malt agar and on various concentrations of hot-water-soluble extracts obtained from the heartwood of air-seasoned western yellow pine. Nos. 1, 2, 3, 4, and 5 represent the growth on concentrations of the extracts of 10, 25, 50, 75, and 100 per cent, respectively.

The growth of the fungus on the concentrations of the various water-soluble extracts and also the percentage of retardation are tabulated in table 1. The curves in figure 1 are based on the growth data in table 1. The graph shows the relative toxicity of each type of extract as removed from the wood of Pondosa pine.

Of all the extracts, the hot-treated air-seasoned heartwood extract was the most toxic to *Lenzites sepiaria*. The 10 per cent concentration showed a retardation of 41 per cent, and the 25, 50, and 75 per cent concentrations showed a total inhibition of the growth of the fungus. The cold-treated kiln-dried sapwood showed the least toxicity to the fungus.

TABLE 1.—*Growth and percentage retardation of Lenzites sepiaria on the various concentrations of the water-soluble extracts of Pondosa pine after 22 days*

Water treatment	Type of wood flour from which water-soluble extract was secured	Control	Average growth in Petri dishes expressed in centimeters and percentage retardation of extracts									
			10% Conc.		25% Conc.		50% Conc.		75% Conc.		100% Conc.	
			Gr.	Ret.	gm.	Ret.	gm.	Ret.	gm.	Ret.	gm.	Ret.
Hot	Kiln-dried heartwood	9.2	8.31	10	6.92	25	5.62	39	4.83	47	2.45	73
Cold	"	9.2	8.2	11	8.6	7	6.78	26	6.46	30	0	100[a]
Hot	Kiln-dried sapwood	9.2	8.16	11	6.42	30	5.38	42	4.2	54	0	100[a]
Cold	"	9.2	9.2	0	9.2	0	8.28	10	7.48	19	0	100[a]
Hot	Air-seasoned heartwood	9.2	5.42	41	0	100[a]	0	100[a]	0	100[a]	0	100[b]
Cold	"	9.2	8.8	4	8.52	8	7.75	16	7.12	23	4.92	47
Hot	Air-seasoned sapwood	9.2	7.86	15	5.86	36	0	100[a]	0	100[a]	0	100[b]
Cold	"	9.2	8.74	5	6.98	24	5.62	39	4.48	51	0	100[a]

[a] Fungus transferred to malt-agar slant recovered.

[b] Fungus transferred to malt-agar slant failed to recover.

The plotted growth data bring out clearly that in every case the hot-water extracts of western yellow pine are more toxic to *Lenzites sepiaria* than the corresponding cold-water extracts.

No very marked difference was noticeable between the toxicity of the heartwood extracts and the corresponding sapwood extracts. This was to be expected, since there is not a great deal of difference in the durability of western yellow pine heartwood and sapwood. The hot-treated air-seasoned-heartwood and the cold-treated kiln-dried-heartwood extracts are more toxic than the corresponding sapwood extracts. The hot-treated kiln-dried-heartwood extract is slightly less toxic than the hot-treated kiln-dried-sapwood extract. This may be accounted for by the fact that, due to trouble in controlling temperatures, the cultures of the hot-treated heartwood extract were grown at a temperature of about 27° C., which is perhaps the optimum temperature for the growth of *Lenzites sepiaria*. After temperature troubles had been adjusted all cultures were grown at 22° C.

The cold-treated air-seasoned-heartwood extract shows a lower toxicity than the cold-treated air-seasoned-sapwood extract. This seems to be at variance with the work done by Hawley, Fleck, and Richards (3) and Sowder (13), in which the heartwood extracts were found to be more toxic in every case than the corresponding sapwood extracts. This reversal can be accounted for only on the assumption that, since the heartwood and sapwood probably did not come from the same tree and since there seems to be so little difference between the toxicity of the heartwood and sapwood water-soluble extracts, the variance may be within the limits of individual characteristics of the wood from different trees.

The work of Hawley, Fleck, and Richards (3) shows that in species where there is little difference between the durability of the heartwood and the sapwood there is also little difference between the toxicity of corresponding heartwood and sapwood extracts.

After plotting the growth data it was noticed that the water-soluble extracts obtained from air-seasoned wood showed a slightly greater toxicity to *Lenzites sepiaria* than the corresponding kiln-dried extracts. This would seem to indicate that the temperatures used in kiln drying western yellow pine may have a slight effect on the chemical properties of the wood. It may be that certain elements in the wood are volatilized.

STUDY OF THE WATER-TREATED WOOD FLOUR

Methods: To determine to what extent *Lenzites sepiaria* can grow on the water-treated wood flour of Pondosa pine, a series of toxicity tests was carried out. The wood flour was first dried to a constant weight at a temperature of 100° C. This required about 48 hours. It was then weighed out in samples of approximately 4 gm. each and in series of 5. The sam-

ples were placed in 150 cc. wide-mouth, flat-bottom extraction flasks of known weights. To find the exact effect of the fungus on the treated wood flour a series of controls of untreated wood flour was made and handled exactly the same as the treated wood-flour samples.

Twenty-five cc. of sterile distilled water were added to each flask. The flasks were next inoculated with a vigorously growing culture of *Lenzites sepiaria* and each flask was then plugged with a cotton plug. To keep the loss of moisture through the plug at a minimum the mouth of each plugged flask was covered with waxed paper and tied securely. The flasks were then placed in a culture chamber and incubated for a period of 5 months at approximately 22° C. After 3 months it was noticed that the cultures were becoming dry, so 5 cc. of sterilized distilled water were added to each flask. Table 2 gives the approximate amount of mycelial growth visible on each culture and the average percentage loss of weight for each type of wood flour.

The percentage loss of weight was computed by dividing the loss in weight of the oven-dry wood flour at the end of the incubation period by the original oven-dry weight of the wood flour.

Discussion of Results: The data of table 2 show that the sapwood of Pondosa pine has less resistance to *Lenzites sepiaria* than the heartwood. The mycelial growth was slightly better on the sapwood cultures than on the heartwood cultures.

The percentage loss of weight based on the original oven-dry weight of the wood flour should be greater for hot-water-treated samples than for the corresponding cold-water-treated samples, because more of the toxic water-soluble material was removed by the hot-water treatment. The loss of weight of the hot-treated kiln-dried heartwood is slightly more than the corresponding cold-water-treated kiln-dried heartwood. The same is true for the hot- and cold-air-seasoned heartwood. Both the hot- and cold-treated air-seasoned sapwood samples and the control show a larger loss of weight than any of the other types of wood flour. The water-soluble extracts of the air-seasoned sapwood gave an acid reaction and, since the growth of fungi is favored by a slightly acid medium, this may account for the good growth of the fungus on the air-seasoned sapwood.

The kiln-dried sapwood cultures show an actual retardation of *Lenzites sepiaria* by the water treatment and do not correlate with the results of the toxicity tests of the water-soluble extracts. The growth of the cultures was irregular. Of the 10 cultures, 3 showed no mycelial growth, 4 showed a little growth, 2 showed medium, and 1 flask had good growth. No reasons can be given that would account for the results obtained in this set of cultures.

TABLE 2.—*Growth of Lenzites sepiaria on the treated wood flour of Pondosa pine*

Water treatment	Wood flour treated	Visible mycelial growth	Percentage loss of weight based on original oven-dry weight	Basis no. of tests
Hot	Kiln-dried heartwood	Medium	12.6	5
Cold	"	Slight	10.5	5
Control	"	"	11.8	5
Hot	Kiln-dried sapwood	"	4.6	5
Cold	"	Medium	10.6	5
Control	"	Good	24.0	5
Hot	Air-seasoned heartwood	Medium	13.1	5
Cold	"	Slight	8.6	5
Control	"	"	14.6	5
Hot	Air-seasoned sapwood	Medium	17.4	5
Cold	"	"	19.9	5
Control	"	"	16.6	5
Total				60

The toxicity of the water-soluble extracts of Pondosa pine is low, and, to secure reliable results from toxicity experiments with hot-and cold-water-treated wood flour, it will be necessary to run a much larger series of tests than it has been possible to run in the preparation of the data for this paper. It would also be advisable to incubate the wood-flour cultures for a longer period than time allowed in this study.

SUMMARY

1. The hot-water extracts of Pondosa pine are more toxic to *Lenzites sepiaria* than corresponding cold-water extracts.

2. In general, the water-soluble-heartwood extracts seem to be slightly more toxic than the corresponding sapwood extracts, but no pronounced difference in the relative toxicity of the two types of extracts was noticed.

3. The water-soluble extracts obtained from air-seasoned material were more toxic to the fungus used than corresponding water-soluble extracts obtained from kiln-dried material. This would indicate a loss of certain volatile materials toxic to *L. sepiaria* brought about by the temperatures used in kiln drying Pondosa pine.

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SPOKANE, WASH.

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HETEROTHALLISM IN PHYTOPHTHORA[1]

LEON H. LEONIAN

Ashby (1) was the first to observe that when a strain of *Phytophthora faberi* Maubl. was grown with a strain of *P. palmivora* Butler or of *P. parasitica* Dastur, oospores developed, while no such bodies formed when it was grown alone. He suggested the possibility of heterothallism in *P. faberi*. Gadd (8) substantiated Ashby's findings and strongly subscribed to the theory of heterothallism. However, Lester-Smith (10) discarded the possibility of heterothallism and suggested that oospore production in paired cultures was due to a biochemical stimulation of the one strain by the other. Ashby (3) in a later work inclines towards the hypothesis advanced by Lester-Smith and subordinates heterothallism to autogamy; he considers chemical changes caused by the association of two different strains as being the chief underlying factor in the production of the sexual bodies. Narasimhan (11) has recently demonstrated that of the 7 Phytophthora cultures (presumably *P. palmivora*) collected by him and tested under laboratory conditions, 4 belonged to one sex and 3 to the other. When grown by themselves or when paired with strains of the same sex, no oospores resulted. Similarly, when *P. parasitica* or *P. meadii* McRae were grown with *P. arecae* (Colem.) Peth., oospores formed, but no such bodies could be found when these cultures were grown by themselves.

All clear-cut cases of heterothallism in Phytophthora have been reported in the *Phytophthora omnivora* group. Clinton's (7) claim that he obtained hybrid oospores by growing *P. infestans* de Bary and *P. phaseoli* Thaxt. together is not conclusive, especially in view of the fact, as shown later in this paper, that the size of oogonia and oospores in the first-generation crosses is controlled by the female strain and that the antheridial strain has little or no influence on the size of these bodies. Furthermore, *P. phaseoli* is homothallic and produces great numbers of oospores in pure cultures; *P. infestans* is also homothallic, at least in so far as our present knowledge goes, but forms its sexual organs only sporadically and quite sparsely. It is, therefore, very difficult, if not impossible, conclusively to demonstrate that hybridization can be brought about between these two species, unless one is able to germinate the oospores and to follow the morphology and the behavior of the second-generation hybrids. The same thing applies to alleged crosses between *P. infestans* and *P. cactorum* (Con. & Leb.) Schroet. If one or the other of any paired culture happens to be homothallic, all claims about hybridization or possible mutual stimulation lose their signifi-

[1] Published with the approval of the Director, West Virginia Agricultural Experiment Station.

cance. Ashby's (2, 3) statement that he observed large oospores when *P. cinnamomi* was grown with *P. parasitica* and (4) with *P. cryptogea* Peth. & Laff. or *P. richardiae* Buism. should not be accepted as sound evidence in support of either heterothallism or the biochemical stimulation theory. All three of these species are homothallic; Ashby (5), himself, has observed oospores in pure cultures of *P. cinnamomi* Rands; all strains of this species, so far examined by the writer, seem to be homothallic. Relative abundance of such bodies is not at all significant, especially when one is advocating the hypothesis that heterothallism is not the controlling factor in oospore production and that the solution of the phenomenon is to be sought in biochemical stimulations.

The present paper is an effort to extend a little farther our knowledge on heterothallism in Phytophthora. Much remains to be clarified and more fundamental research on a larger scale is essential before indisputable conclusions can be proposed.

It was realized at the beginning that no conclusive data could be presented without employing a large number of strains in the experimental work. Accordingly, some 85 cultures of the *Phytophthora omnivira* group, occurring largely in the Tropics, were collected and paired in hundreds of cultures. It soon became evident that variability was as great a factor in the sexual relationships of these fungi as it is in morphological characters and in physiological reactions. Eventually four distinct groups were segregated: 1, homothallic; 2, heterothallic; 3, inconstant; and 4, neutral. The forms of the inconstant group were at times heterothallic and at times neutral; when paired with the proper sex they sometimes formed oospores and sometimes they did not. The neutral forms produced no oospores, regardless of whether they were grown with male or female strains. After the organisms belonging to the first, third, and fourth groups were eliminated, 48 cultures still remained. One-half of these consisted of males and the other half of females. Any of the male strains when mated with any of the female strains gave rise to oogonia, whereas males paired with males and females with females, or else males or females grown by themselves, failed to produce any oogonia.

Oatmeal agar in Petri dishes was used throughout this work. Many other agars were tried and discarded as they failed to induce sexual bodies. It makes no difference how the two pieces of inoculum are planted in the dish; they may be planted together in the same spot or they may be planted any distance apart in the dish; oogonia will form whenever the two hyphae of opposite sexes touch or mix.

The heterothallic strains of Phytophthora here studied are classified in the following table according to their sexuality and origin. In case of some strains there are no data available as to their exact origin and hosts;

however, they are all tropical forms, and there is no doubt concerning their taxonomic status. While all organisms listed in this table belong to *Phy-*

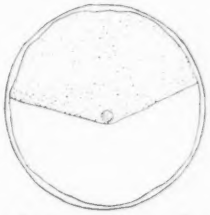

FIG. 1. Sketch of an oatmeal-agar culture of a second-generation transfer, *Phytophthora palmivora* 1 × *P. palmivora* 2. Shaded region represents the oogonia-bearing sector, while the unshaded region consists of a pure culture of *P. palmivora* 1 bearing no oogonia.

TABLE 1.—*Male and female strains of Phytophthora omnivora*[a]

Female strains	Male strains
P. faberi 4, cocoanut	*P. faberi* 1, cocoanut
P. " 5, papaya fruit	*P.* " 2, "
P. " 6, cocoanut	*P.* " 3, "
P. " 7	*P.* " 8
P. " 10	*P.* " 9
P. " 14	*P.* " 11
P. palmivora 1, cocoanut	*P.* " 12
P. " 4, Borassus	*P.* " 13
P. " 9	*P.* " 15
P. parasitica 1, tomato, Morgantown, W. Va.	*P.* " 16
P. " 2	*P. palmivora* 2, cocoanut, Porto Rico
P. " 4, Citrus, Philippines	*P.* " 3, Sabal, Porto Rico
P. " 5, Hibiscus, Java	*P.* " 5, grapefruit, Porto Rico
P. " 7, Bryophyllum, Porto Rico	*P.* " 6, Hevea
P. " 8	*P.* " 7, Ashby's strain
P. " 10	*P.* " 8
P. " 11, Bryophyllum, Bermuda	*P. parasitica* 3, eggplant, Philippines
P. " 13	*P.* " 6, tomato, Porto Rico
P. " 14	*P.* " 9, Vigna, Java
P. " 15—II, III, IV, V, VI, VII	*P.* " 12
P. " 16	*P.* " 15—I
P. " 17	*P.* " 18
P. terrestris, Sherbakoff's strain	*P.* " 19
P. manoana, Sideris's strain	*P. nicotianae*, Holland strain

[a] Most of the organisms listed in this table were secured from C. M. Tucker, Carl Hartley, American Type Culture Collection, and Centraalbureau voor Schimmelcultures, Baarn, Holland.

tophthora omnivora group, their older names are retained here for the sake of convenience in making comparisons.

One interesting thing brought out in the foregoing table is that of the 7 dissociants of *Phytophthora parasitica* 15, 6 are female and 1 is male. As these 7 strains were separated long before their sexual or heterothallic tendencies were tested, the writer has no direct evidence to show the exact sex of the original strain; however, since only 1 strain of the 7 is male, it is safer to assume that the sexual tendency of the original culture was toward femaleness. All 6 of the female strains are sparse oogonia formers, which seems to indicate that their femaleness is not very pronounced; but the 1 male strain has a strong male tendency and when mated with such a prolific female as *P. palmivora* 1, it is able to supply all the antheridia that this female strain needs for its numerous oogonia. It may be stated that the original culture might have been a mixture of two organisms and that *P. parasitica* 15—I was an entirely different culture from the other 6; this is possible but not probable. It still remains to be demonstrated that 2 distinct strains of any given species can be carried together in pure culture

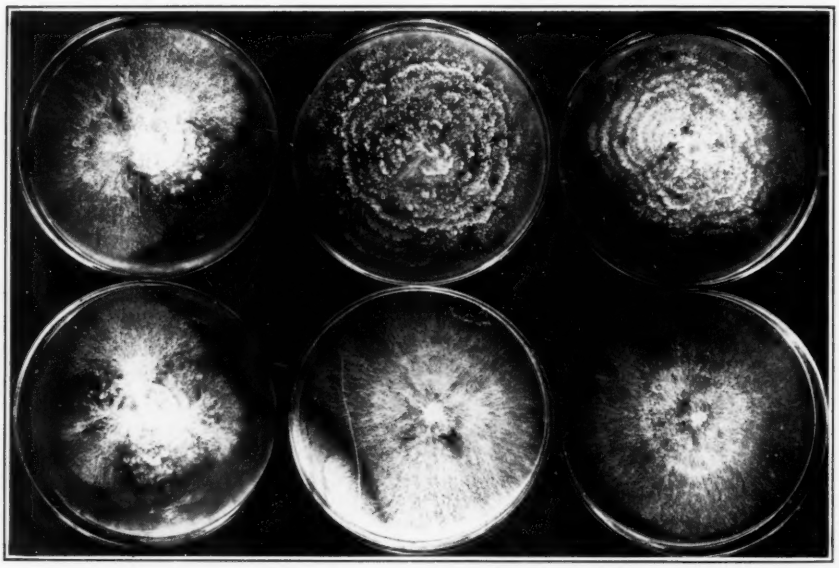

FIG. 2. Upper right, *Phytophthora parasitica* 15; lower right, *P. palmivora* 4; pure cultures showing the growth habit of these two fungi. Upper middle, mixed culture; *P. parasitica* 15 has outgrown *P. palmivora* 4, the latter appearing as a faint growth in the center of the colony; lower middle, *P. palmivora* 4 has outgrown *P. parasitica* 15. Left row, *P. parasitica* 15 and *P. palmivora* 4 splitting apart. In case of the middle two and the left two cultures, transfers were made directly from the oogonia-bearing regions in oatmeal agar, to plates of malt-extract agar.

for any length of time. Usually after 1 or 2 transfers one or the other strain will be left behind. (Figs. 2 and 3.) (Also note similar phenomena observed by the writer (4) in case of *Fusarium moniliforme*.) *Phytophthora parasitica* 15 has been in pure culture for many years and has been transferred from plate to plate and from tube to tube so many times that the likelihood of a simultaneous transfer and subsequent harmonious growth of two different strains of fungi is extremely remote. Furthermore, all seven strains of this fungus show almost identical reactions and many points of close similarity and constitute, in all probability, the different dissociants of the same fungus. Since almost every morphological and physiological characteristic of a given fungus may be materially altered by the dissociation phenomena, there is no reason to suppose that sexuality constitutes an exception.

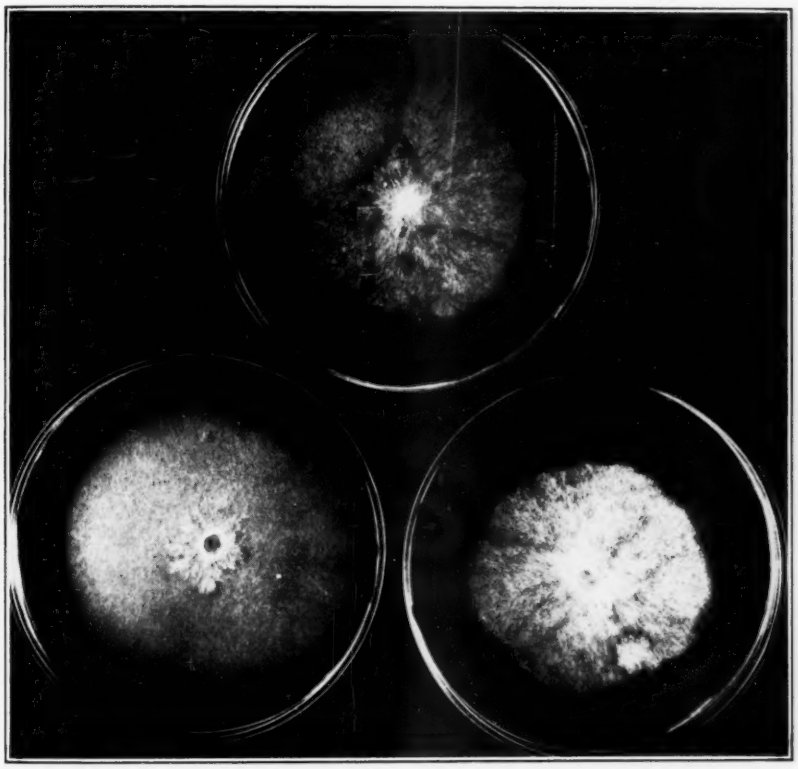

FIG. 3. Second-generation transfers from oogonia-bearing regions on oatmeal agar to malt-extract agar, *Phytophthora faberi* 1 × *P. parasitica* 5. Upper picture, *P. faberi* 1 sectoring away from *P. parasitica* 5. Lower left, *P. faberi* 1 has outgrown *P. parasitica* 5; the latter can be seen as a small growth confined to the center of the colony. Lower right, *P. parasitica* 5 has completely outgrown *P. faberi* 1.

The oogonial and the antheridial strains were identified by the following method: oatmeal-agar plates were poured and sterilized. After the agar hardened, a strip about 1 cm. wide was cut away through the center of the plate, thus leaving a channel that separated the two halves of the oatmeal agar. These two halves were then inoculated by transferring one strain to the one half and the opposite strain to the other half. A sterilized transparent agar consisting of 5 gm. of dry malt extract, ½ gm. each of magnesium sulphate and dihydrogen potassium phosphate, and 20 gm. of agar agar in 1,000 cc. of distilled water was poured into this channel and allowed to harden there. As the hyphae from the two halves of the oatmeal agar grew into this transparent agar, met there and formed their oogonia, the plates were inverted under the microscope, and the oogonial branch was located and then traced to its source. The malt-extract agar alone is unable to induce oogonia; in fact, the opposite sexes often repel each other if grown on this agar. But, when the hyphae were allowed to feed on oatmeal, the carrying-over effect of the substance essential for oogonial production was sufficient to induce the formation of such bodies on the transparent agar. This carrying-over effect has a radius of about 1 cm., beyond which no oogonia form. This was demonstrated by the following method: malt-extract agar was poured in Petri dishes and sterilized; after it hardened,

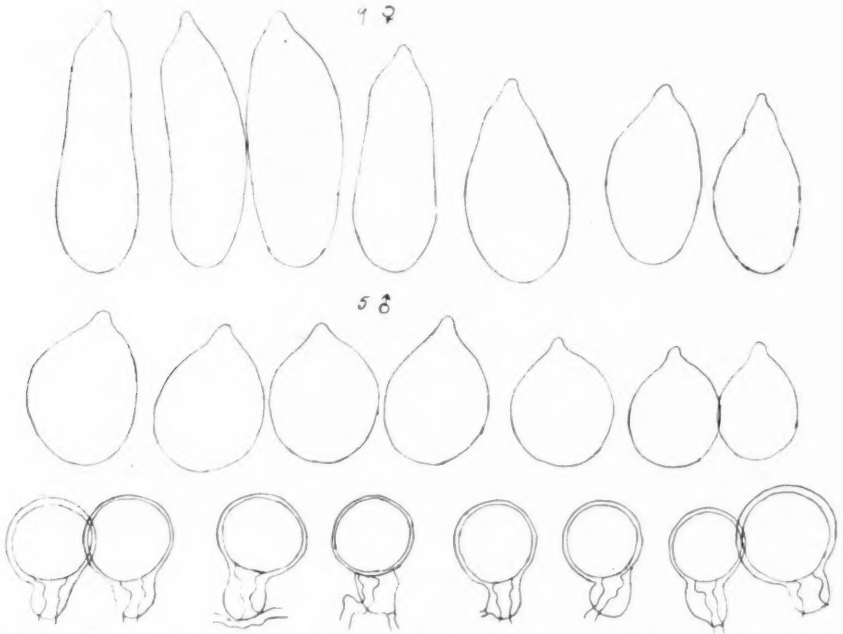

FIG. 4. Upper row, sporangia of *Phytophthora palmivora* 4; middle row, sporangia of *P. parasitica* 15; lower row, oogonia. Drawn to the scale.

large discs were cut away from the center of the plates by means of a cork borer with an opening of 2¼ cm. The vacant circular area thus formed was filled with sterilized oatmeal agar and inoculated with the male and female strains. Oogonia formed not only in the oatmeal agar but also in the surrounding malt-extract agar for a radius of 1 cm. around the oatmeal.

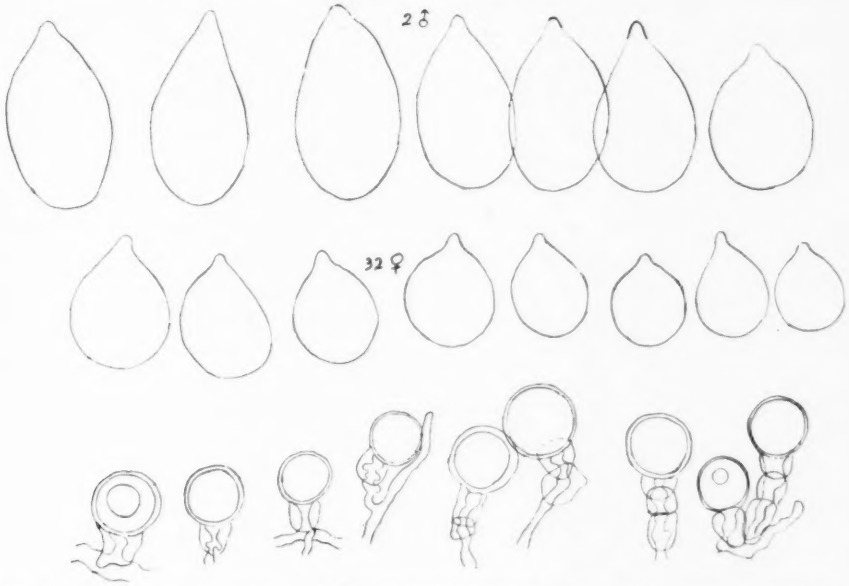

FIG. 5. Upper row, sporangia of *Phytophthora palmivora* 2; middle row, sporangia of *P. parasitica* 14; lower row, oogonia. Note that some of the oogonia possess two and three antheridia in a row.

Aside from the evidence of direct microscopic examination, two indirect methods were used to substantiate the microscopic determination. The size of oogonia is controlled by the female strain; a given female strain, which produces only small oogonia, will continue to do so no matter with what male strain it is mated. For instance, *Phytophthora palmivora* 4, when mated with any of the male strains, forms oogonia that measure 35 μ; whereas, *P. parasitica* 14, mated with the same male strains, will form oogonia that will average not more than 28 μ (Fig. 6). This is indirect but substantial evidence that *P. palmivora* 4 or *P. parasitica* 14 are the oogonia-forming strains and that any strain that can react with these must be male. The relative quantity of oogonial production is another safe indicator of sex. For instance, *P. palmivora* 1, when mated with any of the opposite strains, forms countless oogonia; whereas, *P. parasitica* 2, mated with the same male strains, invariably gives rise to few oogonia. The logical assumption, therefore, is that *P. palmivora* 1 and *P. parasitica* 2

are oogonial strains and all cultures that will react to them to form oospores must be antheridial strains.

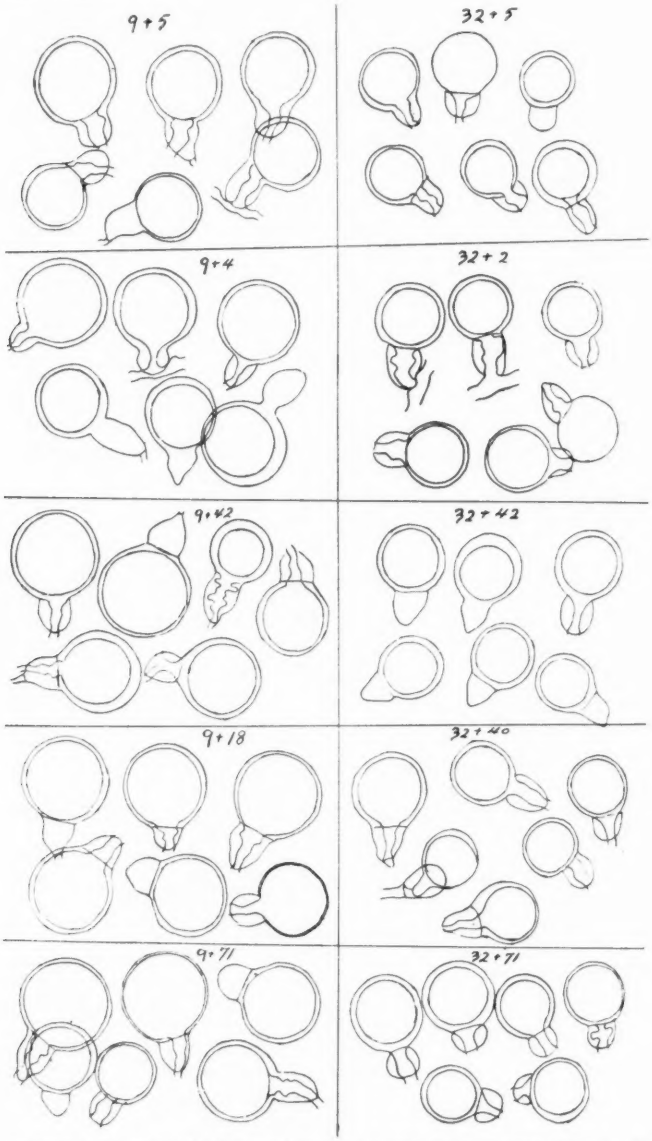

FIG. 6. Oogonia of *Phytophthora palmivora* 4 and of *P. parasitica* 14. Left column, *P. palmivora* 4 mated with *P. faberi* 2 (9×5), *P. faberi* 1 (9×4), *P. faberi* 13 (9×42), *P. palmivora* 6 (9×18), and *P. parasitica* 18 (9×71). Right column, *P. parasitica* 14 mated with *P. faberi* 2 (32×5), *P. palmivora* 2 (32×2), *P. faberi* 13 (32×42), *P. faberi* 11 (32×40), and *P. parasitica* 18 (32×71). Note the uniformly larger-size oogonia of *P. palmivora* 4 and the uniformly smaller-size oogonia of *P. parasitica* 14.

The first sexual bodies to form in paired cultures are to be found along the line where the two opposite hyphae first come together. The oospores, however, do not, by any means, confine themselves to this narrow region but gradually spread out and eventually may cover the entire plate. This indicates that the mycelium of one strain can grow readily into a region already occupied by the hyphae of the opposite strain.

Age of the mycelium is not a limiting factor in oogonial formation; fusion will occur if either young or old hyphae of the opposite sexes are brought together. This was demonstrated by the following method: oatmeal-agar plates were poured and sterilized; some of these were inoculated with the male strain alone, others with the female strain. One week later the entire culture, either male or female, consisting of mycelium and agar, was removed from one plate and inverted over the culture of the opposite sex in another plate; 2 and 3 weeks later the same thing was repeated with the remaining cultures. When examined, oogonia were found in all of the cultures thus treated.

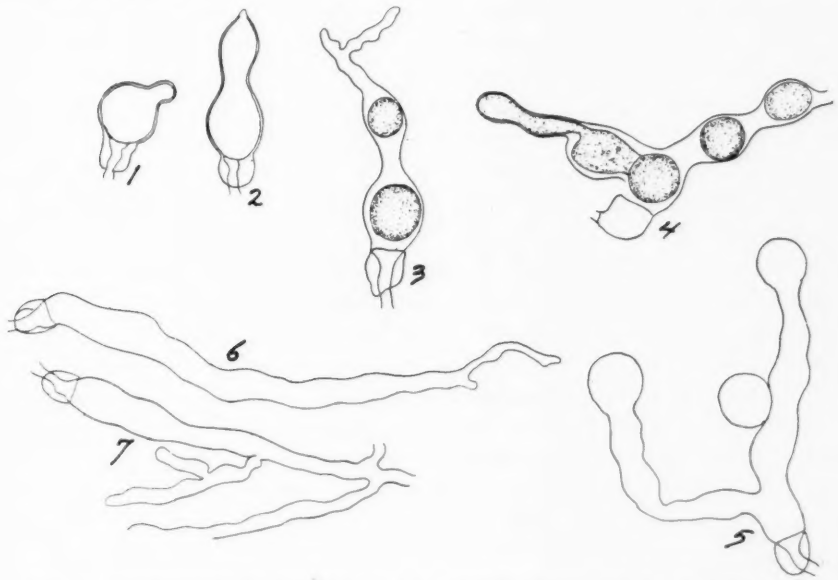

FIG. 7. Germination of oospores of *Phytophthora palmivora* 4. The round bodies in 3 and 4 probably are secondary oospores. The germination occurs *in situ;* only a small percentage of the oospores, however, shows any tendency towards germination.

Although, oogonia formed invariably whenever the two opposite sexes were mated together, transfers made from such cultures did not always give rise to oogonia. This was tested with the most prolific cultures for five generations. In the first-generation transfers the percentage of cultures

having formed no oogonia was quite small. For the second generation transfers selections were made from cultures which showed the greatest abundance of sexual bodies. This was continued until the fifth generation. It was noted that with each successive transfer the percentage of cultures showing no oogonia increased until, after the fourth-generation transfer, no oospores formed, even though a microscopic examination of the inoculum in each case showed an abundance of plump, normal-looking oospores. Some of the cultures manifested a sharp sectoring (Fig. 1), one part of the colony being full of oospores and the other part showing none. Transfers made from this latter region gave rise to a pure colony of the female strain which, when subsequently mated with the same male strain, gave rise to an abundance of oogonia. This shows that constant association of the two sexes tends to bring about a temporary sexual incompatibility, so that, despite the intimate presence of the two sexes, no fusion takes place; there may even be an actual repulsion. Such a behavior may seem strange if we were to regard sexuality as a constantly vital and fundamental phenomenon in these organisms; but if we regard chemotropism as the basis of all heterothallic and parasitic affinities, the foregoing behavior of the sexes will not seem quite so mystifying. Burgeff (6) has already advanced the hypothesis that certain species of Mucorales have become parasitic upon other species as a result of attempts to bring about hybridization. Assuming, therefore, that heterothallism can be explained on the grounds of chemotropic affinities or parasitic tendencies, it will not be difficult to understand that sometime in the relationship of the two sexes periodic changes in the protoplasm of one or the other sex will bring about resistance against the advances of the opposite sex, so that sexual affinities are submerged and a temporary sexual neutrality is brought about. The constant asociation of the opposite sex will serve to uphold this neutrality; but when the two sexes are separated the former affinities once more come to the fore. This explanation may sound fanciful, but it has an analogous case in the relationship of bacteria and bacteriophage where a constant association brings about a temporary immunity in bacteria towards the bacteriophage, but when this association is terminated the immunity disappears.

THE EFFECT OF DYES ON THE GROWTH REACTION OF DIFFERENT SEXES

In order to find out if there is any correlation between sexuality and the growth reaction of the different strains of Phytophthora used in this work, two dyes, crystal violet and malachite green, were used in minute quantities. A nutrient solution was prepared consisting of the following ingredients: 2 gm. of protease peptone, 0.5 gm. each of dihydrogen potassium phosphate and magnesium sulphate, 0.2 gm. succinic acid, 5 gm. of

dextrose, and 1,000 cc. of distilled water. This solution was separated into two lots; to one was added enough crystal violet to give a proportion of 1 part of the dye in 1,000,000 parts of the nutrient solution. To the second lot was added enough malachite green to give a proportion of 1 part of the dye in 2,000,000 parts of the nutrient solution. These were tubed, 5 cc. of the solution in each tube, and sterilized. Transfers were made to these tubes from vigorously growing colonies in Petri dishes by cutting inoculum discs from the outermost edge of the colony by means of a cork borer of 4-mm. bore. Readings were made 2 weeks later. The results are given in tables 2, 3, and 4.

The foregoing 23 organisms were the only ones unable to grow in the presence of 1 part of crystal violet in 1,000,000 parts of the nutrient solution.

TABLE 2.—*Organisms unable to grow in the presence of 1: 1,000,000 crystal violet*

Female strains	Male strains
P. faberi 4	*P. faberi* 1
P. " 5	*P.* " 3
P. " 6	*P.* " 8
P. " 7	*P.* " 11
P. " 10	*P.* " 12
P. " 14	*P.* " 13
P. palmivora 1	*P.* " 15
P. " 4	*P.* " 16
P. parasitica 15—III	*P. palmivora* 5
P. " 16	*P.* " 6
P. manoana	*P.* " 7
	P. " 8

TABLE 3.—*Organisms unable to grow in the presence of 1: 2,000,000 malachite green*

Female strains	Male strains
P. faberi 4	*P. faberi* 3
P. " 5	*P.* " 8
P. " 6	*P.* " 9
P. " 7	*P.* " 12
P. " 10	*P.* " 13
P. palmivora 1	*P.* " 15
P. " 4	*P.* " 16
P. " 9	*P. palmivora* 2
P. parasitica 5	*P.* " 6
P. " 16	*P.* " 7
	P. " 8
	P. parasitica 15—II
	P. " 18

TABLE 4.—*Organisms able to grow in the presence of 1: 2,000,000 malachite green*

Female strains	Male strains
P. faberi 14	*P. faberi* 1
P. parasitica 2	*P. palmivora* 3
P. " 4	*P. nicotianae*
P. " 7	*P. parasitica* 6
P. " 8	*P.* " 9
P. " 10	*P.* " 12
P. " 13	*P.* " 19
P. " 14	
P. " 15, I, II, III, IV, V, VI, VII	
P. " 17	
P. terrestris	

The foregoing three tables show that there is no correlation between sexuality and growth reaction of these organisms in the presence of minute quantities of crystal violet and malachite green. They also show that, generally speaking, *Phytophthora parasitica* type strains are more resistant to dyes than *P. faberi* or *P. palmivora* types. However, it should be borne in mind that these two types are often interchangeable, whereby *P. faberi* type may dissociate into *P. parasitica* type or *vice versa*.

DISCUSSION

A review of the observations by other workers as well as of the results shown in this paper leads to the conclusion that heterothallism, rather than any biochemical stimuli due to associations, is to be considered the chief factor involved in the production of oospores in the *Phytophthora omnivora* group. The chemical-stimulus hypothesis seems rather far fetched, and Narasimhan is correct in stating that "this view does not explain why oospores are produced only when certain strains are paired" and no others; nor does it explain the behavior of sexually neutral strains, either temporary or permanent. Lester-Smith's theory concerning "a low rate of metabolism characterized by low water content and different ratio of food materials" should be operative just as well when only male strains are paired together or when only female strains are grown in mixed culture. One should be able to obtain oospores by pairing other species of Phytophthora, and species of other genera with either the antheridial or oogonial strains of these heterothallic fungi. Until this is done or until oospores are produced readily and abundantly by the mere manipulation of the environmental conditions, heterothallism will remain the only logical explanation. However, the writer does not believe that the heterothallic

strains discussed in this paper are strictly unisexual. The potentialities of both sexes may be present in some or all of these strains. One sex, however, is so much more dominant and the other so deeply submerged that the different strains behave as unisexual organisms. Just as numerous other characteristics may be carried in the protoplasm for a long time without being manifested and may only occasionally reassert themselves by sectoring away from the old colony, so, the opposite sex may be crowded away and inactivated. The curious periodicity in the oospore production of *P. infestans* and other species strengthens this view. Similarly, the behavior of the inconstant strains discussed in this paper, and the apparent sexual neutrality, even when the opposite sexes are in intimate contact, seem to indicate that sexuality is not a definitely fixed character but that it may depend on both internal and external environmental factors as well as on the introduction of the opposite strains.

Once the presence of heterothallism is definitely established, the ardent taxonomist is going to be confronted by a most disconcerting situation because then many of his so-called species will begin to totter and fall. It is going to make no difference whether we call the phenomenon heterothallism or hybridization, the ultimate result will be the relegation into discard of a number of species that have cluttered the literature. Narasimhan (11) states that "in the case of oospore formation induced by the union of *P. arecae* with *P. parasitica*, it would appear more probable that we have to do with hybridization." When two cultures of Phytophthora, each possessing some more or less definite morphological distinction, unite to give rise to oogonia, such a union is more likely to be considered as true hybridization between two species rather than as heterothallism between male and female strains of the same species. A glance at figures 4 and 5 is sufficient to substantiate such a consideration. When a strain, the largest sporangia of which average not more than 45 μ, mates with another strain whose sporangia average 85 or 90 μ, the orthodox morphologist or the less experienced observer will invariably subscribe to the hybridization theory. It is, however, a well-known but poorly appreciated fact that sporangia are extremely unstable in their morphology and exhibit startlingly radical modifications when the parent cultures dissociate into variants. It is, indeed, very unfortunate that the significance of dissociations in taxonomy is not fully appreciated and is lightly waived aside by Ashby and others by the statement that an avoidance of the use of the kinds of nutrient media which the writer has employed in his work will do away with such dissociations. Artificial suppression of some potential characteristic of a fungus is not going to assure us that such potentialities will not constantly manifest themselves under the more complex natural conditions. We should, on the contrary, stimulate all efforts towards the tracing of the

full sphere of variability of any given organism. Otherwise, every dissociant that may appear in nature will be considered a new species or, at least, a new variety. Even if for the sake of upholding the sanctity of a hollow name we were to assume that *Phytophthora palmivora, P. faberi, P. parasitica, P. terrestris* Sherb., *P. nicotianae* B. de H., *P. manoana, P. meadii,* and *P. arecae* are all true species, and when crossed in cultures the resulting oospores are true hybrids, we would still be unable to get away from the great taxonomic tangle that would result when such oospores germinate to give rise to their progenies. What would then be a pure species, what would be a hybrid, and what a new species? On what ground would they be separated and identified and who would attempt such a task? If we were to admit that any of the organisms listed in the foregoing table are *bona fide* species of one kind and that any other organism that will mate with any of the 48 cultures listed here may be another good species, then any one who happens to isolate a given strain of Phytophthora would have the dilemma of a possible hybrid staring him in the face. Any normal fluctuation or any dissociative phenomena would appear like segregations of hybrid characters, and attempts properly to classify such an organism would resolve into a hopeless task.

No two of the foregoing 48 organisms are identical. In growth habit, in the nature of submerged hyphae, and in the size and shape of sporangia, not to mention host relationships and numerous physiological dissimilarities, these organisms show some striking differences. What sort of progenies will result from the union of such strains? Since mating occurs with the greatest of ease under laboratory conditions, there is no reason to suppose that the same thing will not take place in nature. The extreme variability manifested by the *Phytophthora omnivora* group can be traced directly to this ready-mating habit. The writer has always maintained that such unstable characters as size and shape of sporangia are not of sufficient specific value and that the specific unit can be fashioned only from the combination of both morphological and physiological characters of not only one but of dozens of "type species." But even such a practice will lead us astray unless we be willing to allow a wide margin of variability and to build our species concept on a generously flexible basis. Otherwise, if we insist on looking for some minor and even major differences between the different strains and on elevating such differences to the rank of specific characters, then there will be a never-ending confusion. No two organisms are absolutely alike, not even when they originate from the same spore. There are too many cases showing that single-spore cultures give rise to amazingly different dissociants. A more tolerant spirit in taxonomy is the only guide to freedom from the rapidly complicating mycological catacombs.

SUMMARY

1. Some 85 cultures of *Phytophthora omnivora* were tested. It was found that 48 of these were heterothallic, equally divided into males and females, while the remainder were classified into inconstant forms that showed sexual reactions at one time and no reaction at other times, homothallic forms requiring no other strain for the production of their oospores, and neutral forms showing no reaction no matter in what combinations they were mated.

2. Once produced, oogonia cannot be perpetuated indefinitely through the continual association of the two mating forms, and in about five generations no such bodies may form, despite the presence of both strains. Even in the same colony the male and the female strains may separate from each other, despite the most favorable conditions for copulation.

3. There is no correlation between sexuality and ability to tolerate certain concentrations of dyes.

4. None of the 48 strains should be considered a distinct species or even a variety. The phenomenon here exhibited is heterothallism and not hybridization. To admit the possibility of hybridization is to go towards greater taxonomic difficulties.

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REPORT OF THE FOURTH ANNUAL COTTON-ROOT-ROT CONFERENCE

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The fourth annual conference of workers who are engaged in the study of the cotton root rot caused by *Phymatotrichum omnivorum* (Shear) Duggar was held at College Station, Texas, on January 19 and 20, 1931. This conference, which is part of the cooperative attack on the root-rot problem by the United States Department of Agriculture and the Texas Agricultural Experiment Station, affords a yearly opportunity for the prompt presentation of results secured during the previous year at the many laboratories and field stations where work on the problem is under way. The 46 papers presented at this conference included results from 6 laboratories, as well as field and plat studies from 8 stations. A total of 34 plant pathologists, soil chemists, agronomists, botanists, and horticulturists took part in the discussions. Of this group, 18 devote full time to work with root rot, while the remainder are connected with it on a part-time basis or in an administrative or advisory capacity.

Director A. B. Conner of the Texas station and Dr. Oswald Schreiner of the United States Department of Agriculture presided at the various sessions. Following an address by Director Conner on the purposes of the conference, the reports of experimental work were presented; these are summarized below in their order on the program.

LIFE HISTORY AND RELATED STUDIES

Physiologic specialization of the fungus.—D. C. Neal, Bureau of Plant Industry, reported that 6 isolations of *Phymatotrichum omnivorum* from Texas material showed negligible differences in cultural characteristics on artificial media or in soil cultures, while 1 isolation from Arizona was consistently light-yellow to pale-yellow instead of ochraceous to buff. One isolation, apparently attenuated after prolonged growth on artificial media, lost the capacity to produce strand hyphae or sclerotia and yielded only fine white strands and fluffy mycelial growth. Study of the reactions of the various strains to temperature, moisture, and acid or alkali tolerance, showed that optimum conditions for growth were similar for the entire group.

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Physiologic forms differing widely in cultural characteristics were described by W. N. Ezekiel and J. J. Taubenhaus, Texas station. On potato-dextrose agar and synthetic media, those strains assigned tentatively to form 1 produce abundant aerial growth, typical buff strands, and sclerotia, while strains belonging to form 4 grow more sparsely, remain white even in old cultures, produce few strands with typical acicular branches, and do not produce sclerotia on any substrata yet tried. Forms 2 and 3 have intermediate characteristics. The forms have retained their distinctive characteristics in culture for 2 years. They have not yet been tested for possible differences in pathogenicity. Simultaneous inoculations of flask cultures with all possible combinations of 5 of the strains have failed to reveal any evidence of heterothallism in the fungus or any suggestion of the nature of its possible perfect stage.

Sclerotia and strands.—Neal reported that in soil of pH 6.8–7.0, collected in the Mississippi Delta, inoculation with the fungus resulted in good growth and sclerotia production, suggesting that this disease might prove serious in the Mississippi Valley Cotton Belt, if once introduced. Corroborating previous results (3), the optimum moisture requirement for sclerotia production in Wilson-clay-soil cultures was found to be between 30 and 35 per cent (dry basis). The average time for sclerotia formation, with the isolations studied, was 13 days. In soil cultures in the laboratory, approximately 95 per cent of sclerotia were still viable after 13 months, and a few after 22 months. In bottles buried, respectively, 24 and 30 inches deep in the field, no viable sclerotia were found after 8 and 9 months. Sclerotia exposed in covered Petri dishes and in calcium chloride desiccators, at room temperature (24° C.), were viable after 3¼ hours but not after 4 hours. Exposure of sclerotia buried in moist, Wilson-clay soil in small boxes, to electric currents of 250 milliamp.–93 volts, and of 1 amp.–278 volts, a.c., respectively, for 5, 10, and 15 minutes, did not affect subsequent germination of sclerotia (6, 7).

Taubenhaus and Ezekiel described laboratory experiments in which sclerotia were produced in soil chambers from naturally infected cotton-root inoculum brought in from the field at monthly intervals from January through December. In a field plat, previously free of root rot, sclerotia were found as early as August 10, coincident with the death of plants inoculated only 39 days before. Sclerotia were found (9) in the field in 13 counties of Texas; in soils of 14 different types; and in fields of infected cotton, sweet potatoes, garden and sugar beets, carrots, okra, and figs.

B. F. Dana, Texas station, found in field plats at Temple, Texas, viable sclerotia that had survived a fallow period of more than 2 years. Strand sclerotia, which persisted in soil fallowed for 18 months after a cotton crop, were found most abundantly at depths of 12–18 inches. Strand sclerotia

from the field were used in successful inoculations of cotton plants. Sclerotia from the field survived air drying in the laboratory for 49 days in soil that dried to 8 per cent moisture by the end of the period, but sclerotia from the same lot did not survive 7 days' drying on filter paper. S. E. Wolff, Texas station, found large numbers of sclerotia in an excavation in virgin prairie (Houston black clay soil), at 3–21 inches deep, but the majority at 4 inches deep, and associated with the roots of infected, perennial, dicotyledonous plants. No infection was found on grass roots. He observed spore mats in the prairie also.

In experiments on the effect of cold on longevity of the fungus, Ezekiel and Taubenhaus used flask cultures containing masses of sclerotia that had been produced therein, agar slants that had just been seeded with individual sclerotia, and young cultures derived from sclerotia. The cultures were stored in a freezing compartment at $-13°$ to $-14°$ C., and periodic transfers showed that both the sclerotia and the cultures were still viable after 24 hours but not after 39 hours. In similar series at 5° to 6° C., there was no loss of viability after 50 days.

Spore stages.—Taubenhaus and Ezekiel reported that inoculations of susceptible plants with the Phymatotrichum spores proved unsuccessful during 1930, as previously. A Hydnum developed profusely not only along the sides of holes dug near cotton plants affected with root rot but also in holes dug in an area of the field where root rot had not been found for at least 5 years. Cotton plants were inoculated with masses of the spines containing many basidiospores, and with cultures, but no infection resulted (9), suggesting that this Hydnum is probably not a stage of *Phymatotrichum omnivorum*. Dana noted the abundant development of the Phymatotrichum stage in Houston, Catalpa, and Crawford soil areas. Most of the spore mats were found on freshly cut banks of newly graded roads, but exposure to full sunlight did not prevent development so long as the surface of the ground remained moist.

Studies on nutritional requirements of Phymatotrichum omnivorum in artificial culture were presented by Ezekiel, Taubenhaus, and J. F. Fudge, Texas station. The fungus grows in synthetic media, even the sclerotial stage developing in cultures in which the source of nitrogen was ammonium nitrate and the source of carbon was dextrose. At a temperature of 28–29° C., growth curves reached a peak in 5 weeks with a substratum high in dextrose and in 3 weeks with one of lower dextrose content, the media becoming increasingly acid as colonies increased in weight but tending toward alkalinity with later degeneration of the mycelium. In media adjusted with phosphoric acid and potassium hydroxide, respectively, growth was prevented at pH 3 but not at pH 9, the greatest growth occurring in the somewhat alkaline substrata. Requirements for growth are a source of nitrogen,

which may be either organic or inorganic, a source of carbon, phosphate, potassium and magnesium or calcium, and possibly minute amounts of other materials (5).

Host plants.—Wolff and Dana reported as additions to the host list 32 noncultivated species and 35 species and varieties of ornamental plants. Examination at biweekly intervals of representative large populations of some winter annual plants, taken from known root-rot areas, showed 32 per cent infection on *Sitilias multicaulis* Greene on May 3, 1930, and 70 per cent on May 16, 4 per cent infection of *Hamosa Nuttalliana* Rydb. on May 16, but none on *Vicia leavenworthii* Torr. & Gray. Ezekiel and Taubenhaus reported that the guayule, *Parthenium argentatum* Gray an American rubber plant, is susceptible to root rot.

Taubenhaus and Ezekiel (8) summarized inoculation experiments with many monocotyledons, including corn and numerous liliaceous plants, none of which became infected, although the interplanted cotton, okra, and carrot plants succumbed to root rot. Black lesions on the roots of many of the grass plants were cultured and invariably yielded other organisms instead of *Phymatotrichum omnivorum*.

Further studies on the physiologic basis of resistance (5) were given by Ezekiel, Taubenhaus, and Fudge. *Phymatotrichum omnivorum* cultures averaged 5 times as much dry weight of mycelium in nondiluted, autoclaved extracts from roots of cotton plants (susceptible) as in corresponding extracts from roots of corn plants (resistant), and the fungus growth was better also in various dilutions of cotton extracts than in dilutions of corn extracts.

Some effects of root rot on host plants.—Ezekiel and Taubenhaus reported that leaves of cotton plants with root rot were 1° to 6° F., averaging 3° F., warmer than those of normal plants. Normal leaves were usually cooler than air temperature, while leaves of plants affected with root rot were only slightly cooler or often warmer than air temperature (4). Periodic analyses of roots of cotton plants following infection with root rot showed decrease in the percentage content of sugars, with progressive increase in water-insoluble proteins.

Overwintering on live infected roots and cyclic periodicity of root rot.—Taubenhaus and Ezekiel reported successful inoculations of plants in July, 1930, with naturally infected, overwintered 1929 cotton roots, and discussed the relation of overwintering of root rot on roots to the cyclic periodicity of the disease under field conditions (9). The fungus was found on the roots of plants during certain years, even though the tops of all the plants appeared normal.

H. C. McNamara, Bureau of Plant Industry, described the reappearance of root rot in continuous cotton fields, in which the disease apparently had died out, since no plants had died there during the previous 4 years.

Dana and H. E. Rea, Texas station, found that the date of appearance of root rot and the loss from the disease were affected only slightly by differences in the date of planting cotton (2).

STUDIES RELATING TO CONTROL

Occurrence of root rot as related to soil conditions.—Taubenhaus and Ezekiel pointed out that root rot has now been found in soils of 30 series (9) and can no longer be considered peculiar to the Houston black clay soils. Root rot has not been found in the field on plants growing in definitely acid soils.

Progress of studies by the Bureau of Chemistry and Soils on the chemical characteristics of the soils of central Texas was outlined by Paul R. Dawson and E. R. Collins. Complete analyses have been made of a large number of soils. No outstanding chemical factor universally correlated with the presence or absence of root rot has as yet been disclosed; however, in most instances, heavily infested areas are calcareous and alkaline (pH 7–8.3), while root rot is less prevalent or absent in noncalcareous soils of neutral or slightly acid reaction (down to pH 6.0).

Dana and Henry Dunlavy, Texas station, discussed local variations in the soil as related to natural occurrence of root rot. A deep Houston black clay soil, averaging 30.5 per cent soil moisture, appeared more favorable for root rot than a shallower Houston clay, which averaged only 21.2 per cent during the same period. Along a sloping field, of which the flat upper part is Houston black clay, while the main slope is eroded to Houston clay, less root rot occurred in each of 3 years in the Houston-clay part of the field, in which the soil is lower in colloidal content, organic matter, and moisture-holding capacity.

Taubenhaus, Ezekiel, and Fudge reported that continuation during 1930 of the studies on the relation of the soil reaction to incidence and overwintering of root rot gave results agreeing with previous reports (3). Root rot was worse on cotton plants grown in neutral and alkaline soils, with less damage in soils below pH 6.3, and little or none in soil at pH 5.6 or lower. Preliminary studies with crops indicated good growth with cotton, for instance, in soil acid enough to impede the development of root rot. Fudge summarized some chemical studies on the nature of basicity in soils.

Subsoiling experiments, conducted independently by a number of workers, again yielded promising results. Dawson and McNamara reported that in a field planted, in 1929, to sorghum, a plat was subsoiled twice during the dry weather of August; cotton was grown in this field in 1930 with only 1.5 per cent root rot in the subsoiled part of the field, which was separated by a sharp line of demarcation from the control, nonsubsoiled area in which 27 per cent of the plants succumbed to root rot. Neal found a reduction in visible symptoms of the disease in subsoiled plats in 4 different localities

but emphasized the presence of deep-seated infection on the roots of many of the plants. Dunlavy and Dana conducted subsoiling tests on 4 acres and found a reduction of root rot in each subsoiled plat, the reductions amounting to 6 per cent to 48 per cent of the root rot in the respective check plats. There were no consistent differences in yield.

Fertilizers.—Dawson and Howard V. Jordan presented the results of the Bureau of Chemistry and Soils fertilizer experiments conducted in the blackland region of central Texas. As in 1929, increases in yield were obtained from applications of mixtures containing combinations of phosphate and nitrogen. In a number of cases, the increases were profitable from an economic standpoint and in most cases were of such magnitude as to offset losses in yield due to root rot. The more highly phosphatic fertilizers accelerated maturity, thus increasing the yield of the early pickings and suggesting a promising means of evading losses from root rot by such additions. In tests by Rea, Dana, and Dunlavy, some increases in yield were secured by the use of fertilizers at the rate of 400 lbs. or 600 lbs. per acre. Ezekiel and Taubenhaus found that fertilizers applied, in container experiments, at the rate of a ton to the acre produced marked responses in the growth and yield of cotton but did not reduce the incidence or overwintering of root rot. Neal found similarly, in field experiments, that applications of 600 to 1,800 lbs. of fertilizers per acre accelerated maturity of plants and gave increased yields over check plats, tending to counterbalance losses from root rot; however, the amount of root rot was not reduced.

Soil disinfectants.—Neal tested various toxic agents at concentrations of 1:200, immersing sclerotia for 5 to 60 min. In the order of their toxicity, the materials tested were: mercuric chloride, ethyl mercury chloride, chlorophenol nitrophenol mercury, Semesan, copper sulphate, calcium chlorate, and Chlorazene. Additions of 5 per cent of marcasite (crystalline ferric sulphide) to the soil in soil cultures inhibited sclerotia formation; and marcasite applied in field plats at the rate of 7.4 tons per acre apparently reduced root rot.

Comparisons of fungicides in the laboratory by the soil-chamber method previously described (3) were summarized by Ezekiel and Taubenhaus. Among the most toxic materials tested were mercuric chloride, which prevented growth of the fungus at a concentration of 50 ppm. of soil, and Semesan, which inhibited growth at somewhat more than 100 ppm.; while some of the least toxic were aluminum sulphate, copper carbonate, and oxymethylene, which did not greatly affect growth, even at 1,000 ppm.

Dana found that dilutions of 1:80 of sodium, potassium, and calcium chlorates prevented the germination of sclerotia, while dilutions of 1:800 reduced but did not prevent germination.

Field-plat comparisons of a number of disinfectants were presented by Taubenhaus and Ezekiel. The relative quantities of the different materials

needed to reduce incidence and spread of root rot in the field correlated fairly well with the quantities required to inhibit growth in the soil-chamber tests. Some of the organic mercury compounds, such as Semesan, yielded somewhat promising results, while with aluminum sulphate, copper carbonate, manganese sulphate, and iron sulphate, the quantities used produced no apparent effect. W. J. Bach reported that copper sulphate at the rate of 1 lb. per 10 gal. of water, applied around the roots of woody plants, such as grapes, 3 times a year, continued to give good results.

C. J. King, Bureau of Plant Industry, reported that at Indio, California, $1\frac{1}{4}$ per cent formalin was injected by pressure into the sandy soil to a depth of 6 ft., at the rate of 6 gal. per sq. ft. Prior to the treatment, cultures of the fungus on fig and pistache roots had been placed at various depths in the soil. After 3 to 4 weeks, the fungus grew out again from the interior of roots $\frac{3}{4}$ of an inch or more in diameter when these roots were placed in moist chambers, but did not grow out from the smaller roots. In laboratory experiments, longer treatments were necessary to kill the fungus inside of roots than to kill sclerotia.

Rotation and cultivation.—McNamara reported that root rot was greatly reduced in cotton following 2 years of sorghum or 2 or 3 years of clean fallow, as compared with plats in continuous cotton or in clean fallow for a single season. Dunlavy and Dana found that the amount of root rot in cotton grown in 2- and 3-year rotations did not differ significantly from that in plats in continuous cotton. Rea summarized tillage experiments in which the treated plats were bedded and rebedded weekly for periods, respectively, of 12 and 24 months. In some of these tests perennial weeds were not eliminated even after 24 months of tillage. These tillage operations, to the shallow depth of 6 in., did not eliminate root rot, and there were significant reductions in root rot in only a few instances.

Barriers to limit the spread of root rot were discussed by Taubenhaus and Ezekiel, with particular reference to some experiments in plats and in containers. Barriers 6 in. wide and 4 ft. deep, consisting of soil into which 4 per cent sulphur had been incorporated, served to check completely the spread of root rot across plats of cotton. Barriers 1 row wide of crops such as sorghum and corn limited root rot to the inoculated rows of cotton plants, in experimental containers, no spread of the fungus occurring either on the roots of these barrier crops or through the soil to reach cotton plants on the other side of the barriers (9).

McNamara reported that of the barriers tested at Greenville, Texas, under field conditions, open trenches 24 in. deep, proved effective in preventing the advance of the disease and sorghum barriers broke up the uniform advance of the fungus.

Flooding experiments at Iowa Park were reported by Taubenhaus and Ezekiel. Although laboratory experiments indicate that the vegetative

stage of the fungus is no longer viable after submergence in saturated soil for 3 days, flooding field plats for as long as 120 days did not eradicate root rot (10).

Resistant strains and varieties.—Bach found that the Champanel, Mustang, Black Spanish, and *Vitis Chantini* grapes retained their resistance to root rot previously reported (3) and that, in addition, the Dog Ridge, *V. Salonis*, *V. Constancia*, and *V. Berlanderi* grapes appear resistant. The Sour Orange rootstock for citrus still appears highly resistant, inoculations having failed to kill the seedlings. In tests of cover crops for use in citrus groves, *Crotalaria spectabilis* Roth. and *C. striata* DC. and cowpeas proved very susceptible, while *C. incana* L. was somewhat resistant and Sesbania showed sufficient resistance to make it of practical value as a winter cover crop.

With the Turk's cap hibiscus, hackberry, live oak, and pomegranate, well-established plants are highly resistant to root rot, yet in the seedling stage many of the plants may be attacked and a small percentage killed (Taubenhaus and Ezekiel). The seedling plants that recover appear to do so by developing new roots faster than the disease can destroy them, while the fungus apparently is unable to attack the roots of the older plants (1).

Cotton variety tests at Temple Substation were summarized by Rea, Dana, and Dunlavy. A large number of selections and most of the varieties available in the United States have already been tested. During 1930, new introductions to the test were primarily field selections of isolated healthy plants from diseased spots. This work yielded a limited amount of promising material that is to be retested in subsequent years.

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AN ANTHRACNOSE OF LEDUM CAUSED BY A SPECIES OF ELSINOË[1]

S. M. Zeller and J. W. Deremiah[2]

Along the Pacific Coast of Oregon *Ledum glandulosum* Nutt., an attractive evergreen shrub of the Ericaceae, is affected by a very common and serious leaf spot. The senior writer has observed the disease since the spring of 1927. The many collections taken in May and June of 1927 and 1928 gave no clue to the identity of the causal organism. During the winter of 1929–30, however, we examined some older material in the herbarium of the Oregon State Agricultural College. A species of Elsinoë was found on a leaf spot of Ledum collected by G. K. Van Gundia at Manhattan, Tillamook County, July 19, 1915. This suggested that all of our previous collections had been taken before the maturity of the fungus fructifications. This has proved true in collections taken since the first part of July, 1930.

The disease attacks the leaves, the younger branches, flower pedicels, and sometimes the calyx and capsules. The most common and conspicuous expression of the disease, however, is the leaf spot.

The disease has been observed all along the Oregon coast from Brookings, Curry County, to just south of Seaside, Clatsop County. Since the leaf spot is so common within these limits, it perhaps could be found throughout the entire range of the coastal form of *Ledum glandulosum*, and the slight variation of it which Piper has described as *L. columbianum*. On *L. groenlandicum* one specimen from Michigan and one from Washington have been examined.

It is evident that the infection of new leaves is influenced by weather conditions. In situations where fogs frequently are driven in from the ocean the leaf spot makes its first appearance as early as June, but, farther inland, where Ledum is found in bogs at slightly higher elevations, infections on leaves of the current season's growth may not be found until August. On high bluffs overlooking the ocean the spotting is not evident so soon as in lower locations nearby. The disease, however, seems to be as severe on the older foliage in the one location as in the other.

Usually the beauty of the host plant is destroyed by the disease of the leaves and upper branches (Fig. 1, A; Fig. 2, A and B). The size of the leaves differs so greatly that the number of lesions per leaf may not indicate the extent of injury. Some small leaves with a dozen spots may have

[1] Published with the approval of the Director as Technical Paper No. 137 of the Oregon Agricultural Experiment Station.

[2] Mr. Deremiah did the histological work connected with this study.

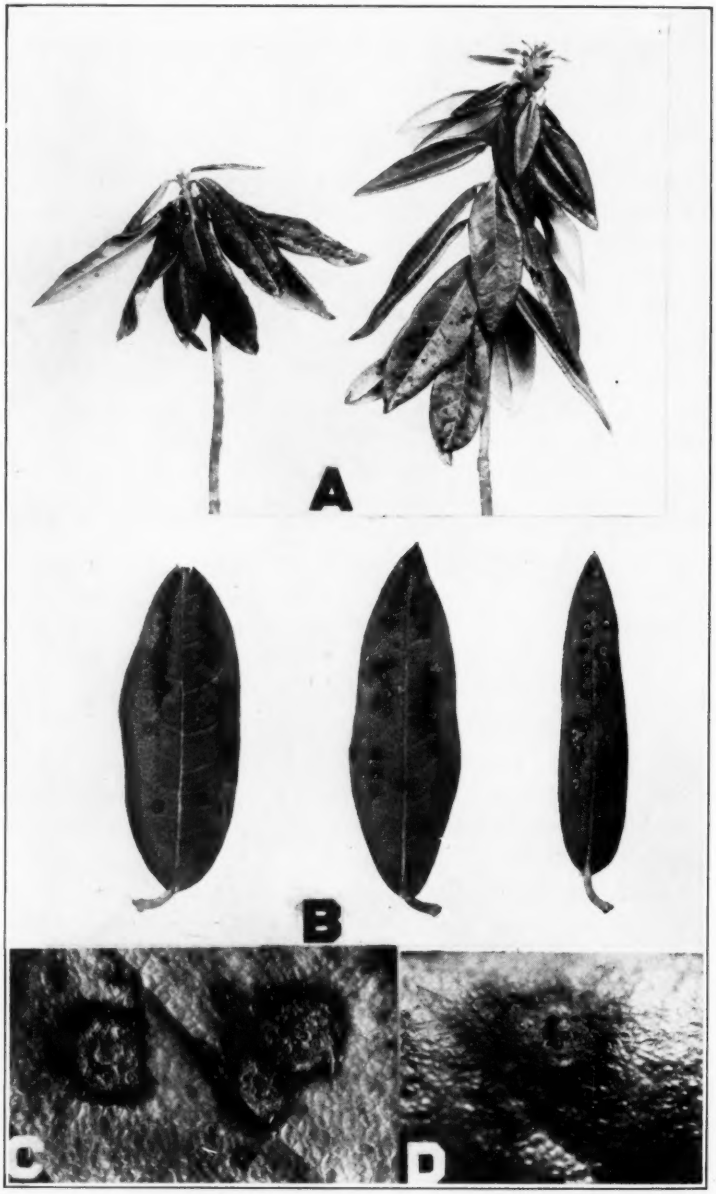

FIG. 1. A. Habit of the branches of *Ledum glandulosum* showing leaves infected by *Elsinoë ledi*. ×2/3. Photo by H. H. Millsap. B. Three leaves showing the characteristic distribution of the leaf spot. ×1. Photo by H. H. Millsap. C. Leaf spots enlarged. ×10. Photo by J. W. Deremiah. D. Leaf spot enlarged to show the fruiting bodies (ascocarps). ×10. Photo by J. W. Deremiah.

50 per cent of the leaf surface destroyed, while another with 35 spots may have two thirds of its surface in apparently healthy condition. From 0 to 47 spots per leaf have been counted (Fig. 1, B).

In some locations the disease causes severe "leaf drop," while, at the same time, in other locations where the spot seems equally severe, there may be no drop. No explanation for this difference is ventured, unless the one location may be drier than the other; but the factors which constitute "physiological dryness" in the sandy bogs where Ledum grows are not easily estimated.

The leaf spot first appears as a tiny circular reddish brown area on the upper surface of the leaf. Spots can be recognized when only $\frac{1}{4}$ to $\frac{1}{3}$ mm. in diameter. The mature ones are from about 1 to 3 mm. in diameter but, where several infections coalesce, they are much larger. As the lesions mature the central part becomes grayish to almost white, while the borders remain reddish brown often with purplish margins (Fig. 1, C). In the central grayish area are to be found the black fruiting bodies of the fungus which is constantly associated with the disease. These fruiting bodies evidently appear within 8 to 12 weeks after the lesions are first distinguishable (Fig. 1, D).

The fruiting bodies break through the upper epidermis of the diseased portion of the leaf. The rupture of the epidermis is usually star-like, having 3 to 5 rays. The fruit bodies of the fungus may take this astral shape or form a flattened hemisphaeroid or elongated pad of dark stromatic tissue.

Vertical sections through the leaf and the fungus were prepared to show the relation of the two. The development of mycelial invasion of the host tissues from the time of infection has not been followed. In mature lesions, however, the fungus hyphae are primarily intercellular, lining the intercellular spaces of the leaf mesophyll and crowding the palisade cells apart. Where the fungous invasion seems complete in the central portion of the leaf spot the crowding of the mycelium has forced the host cells, and, with additional fungous growth, these cells are almost entirely separated, causing almost complete disintegration of the host tissues. At these centers of concentrated fungous growth the fundament of the fruiting bodies pushes up under the cuticle or in some cases raises the whole epidermis. Plugs or palisades of erect hyphae that are cemented into a prosenchyma of somewhat harder consistency bring about the primary rupture of the surface (Fig 2, C). This first concentrated fungous growth is comparable to the ectostroma of some of the Sphaeriales.[3] The general upward growth during this period of development in the interior of the leaf often carries with it

[3] Ruhland, W. Untersuchungen zu einer Morphologie der stroma-bildenden Sphaeriales. Hedwigia 39: 1–79. 1900.

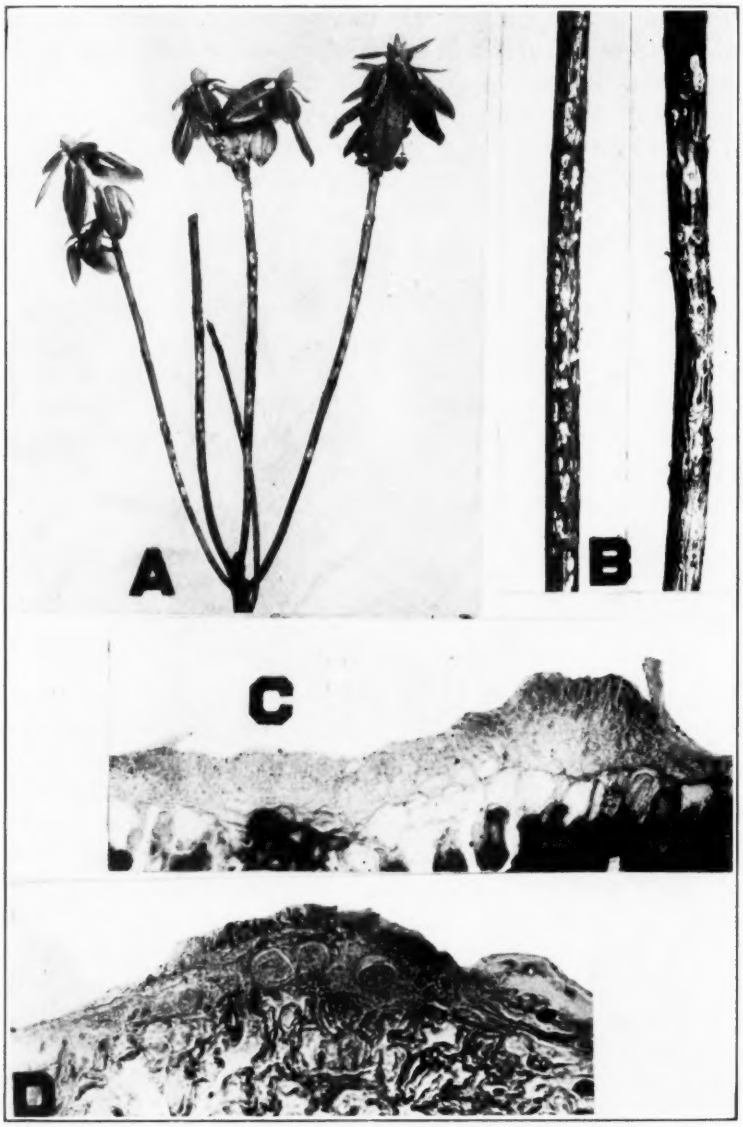

FIG. 2. A. Stems of *Ledum glandulosum* infected by *Elsinoë ledi*. ×2/3. Photo by H. H. Millsap. B. Stems showing anthracnose spots. ×1½. C. Photomicrograph of a vertical section of an acervulus-like, sterile ectostroma showing broken cuticle. The higher darker, raised portion at the right is the palisade of rigid prosenchyma which ruptured the cuticle. The entostroma, or ascocarp, is below and to the left of the ectostroma. ×300. Photo by W. C. Whitaker. D. Photomicrograph of a vertical section of an entostroma (ascocarp) showing three asci and a portion of the ectostroma. ×300. Photo by W. C. Whitaker.

islands of leaf tissue. These may be embodied in the lower part of the mature fruiting body or ascocarp. The mature entostroma or ascocarp is a homogeneous pseudoparenchyma which forms under the original pad of erect hyphae, the latter being sloughed off in a manner closely resembling that in which the acervulus of *Neofabraea malicorticis* (Cordley) Jackson is forced aside by the newly formed ascocarp under it.[4] (Fig. 3.) Some-

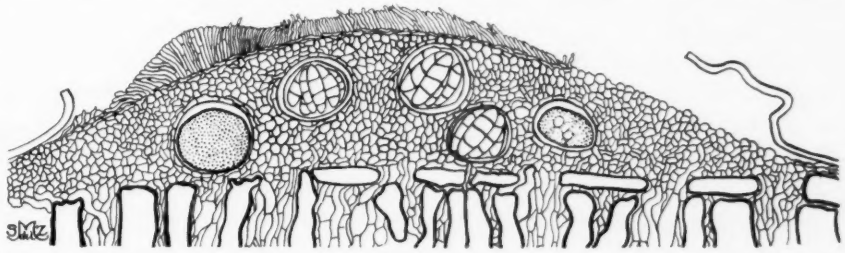

FIG. 3. Drawing showing a vertical section of an entire mature ascocarp illustrating the discarded ectostroma and the ascogenous entostroma. Immature and mature asci usually occur together in an ascocarp. ×500.

times the ectostroma persists more or less loosely attached above or to one side of the mature ascocarp. Even though the ectostroma thus has the appearance and structure of an acervulus, conidia have not been observed in connection with it.[5] The ascocarps occur in various sizes and shapes but the most common semilenticular forms are from 70–195 μ in diameter and have a depth of 28–110 μ. The spherical asci are scattered in monoascoid locules throughout the pseudoparenchyma of the ascocarp. As a rule, the ascogenous locules are near the surface of the structure, more or less in one stratum (Fig. 2, D), but often there is no order of arrangement, the locules being scattered through the pseudoparenchymatous tissue to a depth equaling three to five times the diameter of an ascus. Asci containing 8 spores predominate but those containing 1 to 4 are not uncommon. The asci measure 17–25 x 21–28 μ. The ascospores are ellipsoid to fusoid, often flat on one side, hyaline, mostly 3-septate (seldom 1–2-septate), and measure 12.3–17.7 x 5–6.5 μ.

This fungus belongs to the genus Elsinoë Raciborski, 1900 (Elsinoaceae von Höhnel), which, according to Shear,[6] is synonymous with Plectodiscella,

[4] Jackson, H. S. Apple tree anthracnose. Oreg. Agr. Exp. Sta., Bien. Crop Pest and Hort. Rpt. **1911–1912**: 178–197. 1913. (See Fig. 4, p. 186.)

[5] Since this paper went to press conidia (Sphaceloma) have been found produced by this ectostromatic stage and in a letter Dr. Anna E. Jenkins says leaf lesions from Oregon material show both imperfect stages of the fungus as they are also on the type material.

[6] Shear, C. L. The life history of Sphaceloma ampelinum de Bary. Phytopath. **19**: 673–679. 1929.

Woronichin, 1914. The species found on Ledum is very similar morphologically to *Elsinoë ampelina* (de Bary) Shear, the cause of grape anthracnose, *P. veneta* Burkh., the cause of raspberry and blackberry anthracnose, and also *P. piri*, the cause of a fruit and leaf spot of apple and pear in Europe. The dimensions of the ascospores are similar to those of these species.

The ascocarp, shown in vertical section in figure 2, D, and figure 3, in general, has the regular outline described for *Plectodiscella piri*, but the ascocarp from Ledum is not covered by a sclerotic, placodium-like rind, although the surface is often made up of a darker brown layer of cells with slightly thickened walls. On the other hand, ascocarps of the fungus on Ledum are not all of the type illustrated but are of various shapes, often like the illustrations of those of *P. veneta* or *Elsinoë ampelina*, and are often surmounted by the acervulus-like palisade of the primary ectostroma.

The fungus, constantly associated with the leaf and stem anthracnose reported in this paper, was first described by Peck[7] as *Aulographum ledi*.[8] The description is unmistakably that of the fungus with which we have been dealing, except that in our material the mature ascospores are 3-septate. It was originally discovered on *Ledum groenlandicum* in New York State.

The other species of Elsinoë are on hosts of remote relationship with respect to the Ericaceae. Though the species on Ledum is very similar to the others in morphology, it perhaps should be retained as a separate species until a critical study of the genus is made. The fungus associated with the disease of Ledum is therefore transferred to Elsinoë as **Elsinoë ledi** (Peck) Zeller, n. comb.

The following amended description is based on the material we have examined.

ELSINOË LEDI (Peck) Zeller, n. comb.

Syn. *Aulographum ledi* Peck.

Spots epiphyllous or on stems, circular or coalescing in lines, 1–3 mm. in diameter, at first reddish brown, then whitish gray with reddish brown to purplish margins; *stromata* solitary or gregarious near the center of the spot, circular or irregular, sometimes astral, erumpent, superficially black, pulvinate, 45–200 μ in diameter; *ectostroma* discoid to subconic, of hyaline, erect, palisaded, prosenchyma, rupturing the cuticle or epidermis, bearing

[7] Peck, C. H. Report of the state botanist, 1910. New York State Museum Bul. 150: 23–24. 1911.

[8] We want to express our thanks to Dr. C. L. Shear and Dr. Anna E. Jenkins, who have examined some of the Oregon material and called our attention to *A. ledi* Peck. In a letter under date of February 25, 1931, Dr. Shear says: "An examination of Peck's type shows that it is the same as your Elsinoë."

conidia belonging to the form-genus, Sphaceloma; *entostroma* or ascocarp arising beneath and rupturing the ectostroma which is sloughed off, hyaline interior with brownish surface, pseudoparenchymatous, 70–195 μ in diameter, 28–110 μ high, semi-lenticular to varied in shape, pluriloculate; *locules* subsphaeroid, mostly monoascoid, 19–30 μ in diameter, scattered in the entostroma; *asci* subsphaeroid, 1–8-spored (mostly 4- or 8-spored) hyaline, 17–25 x 21–28 μ; ascospores ellipsoid to fusoid, mostly unilateral, mostly 3-septate (seldom 1–2-septate) 12–17.7 x 5–6.5 μ.

On the leaves, stems, and capsules of *Ledum glandulosum* Nutt., Curry to Clatsop counties, Oregon, and on leaves of *L. groenlandicum* Oeder, Ingham County, Michigan, and King County, Washington.[9]

Specimens examined:

Michigan: Ingham County, Towan's Swamp, East Lansing, May 26, 1895, *A. B. Cordley* (in O. A. C. Herb., 7946).

Washington: King County, north of Seattle, May, 1912, *S. M. Zeller* (in Zeller Herb., 582).

Oregon: Clatsop County, Seaside, July 6, 1928, *S. M. Zeller* & *C. E. Schuster*, (in Zeller Herb., 2199); Coos County, Bandon, June 26, 1928, *S. M. Zeller* (in Zeller Herb., 6774); Lakeside, June 27, 1928, *S. M. Zeller*, (in Zeller Herb., 1759); Marshfield, June 27, 1928, *C. E. Schuster* & *S. M. Zeller*, and Sept. 18, 1930, *L. N. Goodding* (in Zeller Herb., 1668, 7891); Curry County, Brookings, June 24, 1928, *S. M. Zeller* (in Zeller Herb., 6776); Gold Beach, June 26, 1928, *S. M. Zeller*, and July 19, 1930, *L. N. Goodding* (in Zeller Herb., 6775, 7770); Douglas County, June 27, 1928, *C. E. Schuster* & *S. M. Zeller* (in Zeller Herb., 1664); Lane County, Cummins Creek south of Cape Perpetua, June 8, 1929, *S. M. Zeller* (in Zeller Herb., 2664); Lincoln County, Big Creek, January 11, 1931, *S. M. Zeller* (in Zeller Herb., 7882); east of Newport, April, 1927, *S. M. Zeller* (in Zeller Herb., 1765); near Jump-off Joe; north of Newport, Jan. 31, 1931, *Wm. Kessi* (in Zeller Herb., 7896); Waconda Beach, Jan. 11, 1931, *S. M. Zeller* (in Zeller Herb., 7884); near Waldport, July 5, 1930, *S. M. Zeller* (in Zeller Herb., 7769, and O. A. C. Herb., 5548); south of Yaquina John Point, July 30, 1930, *S. M. Zeller* (in Zeller Herb., 7768); Tillamook County, Manhattan, July 19, 1915, *G. K. Van Gundia* (in O. A. C. Herb., 5549).

So far as we are aware *Elsinoë ledi* occurs on Ledum only. There are species of fungi, however, which occur on several different ericaceous hosts.

[9] Dr. C. L. Shear states that there are collections in the U. S. Bureau of Plant Industry on *L. glandulosum* from Mendocino County, Calif., and on *L. groenlandicum* from North Mountain, Pa., Meadowlands, St. Louis County, Minn., and the *type* in the New York State Museum from Fine, St. Lawrence County, N. Y.

One of these, **Cryptostictis arbuti** (Bonar) Zeller, n. comb. (= *Disaeta arbuti* Bonar), frequently occurs in Oregon on *Ledum glandulosum* in close proximity to *E. ledi*. In fact, leaf spots of the two fungi are often found on the same leaf. *Cryptostictis arbuti* was first reported on Arbutus and has been found on Arctostaphylos, and it would not, therefore, be surprising to find *E. ledi* on other ericaceous hosts.

SUMMARY

An anthracnose of the evergreen shrub, *Ledium glandulosum*, is described. It occurs along the Pacific Coast of Oregon and Washington, and one specimen from Michigan on *L. groenlandicum* has been examined. The disease seems to be distributed through the range of the genus Ledum in the United States. Leaf and stem spots are the chief symptoms of the disease. **Elsinoë ledi** (Peck) Zeller, n. comb. (Syn. *Aulographum ledi* Peck) is constantly associated with the disease. The fungus was first described on *L. groenlandicum* from St. Lawrence County, New York. An amended description, with illustrations of the fungus and disease, is included in the present paper.

BLACKLEG OF TOBACCO SEEDLINGS[1]

E. M. JOHNSON AND W. D. VALLEAU

On May 13, 1930, when plants were being pulled for transplanting, the writers found, in a bed of Burley tobacco near Lexington, Kentucky, an area of plants that had a rot which seemed unlike the rots caused by the damping-off fungi. This disease was found in another bed several miles from the first and, later, in several other parts of the first bed. When the cotton was first removed from the bed, the diseased areas were difficult to detect, as the plants, even though almost completely rotted off, were still turgid. The affected areas were found during pulling and could be located without difficulty after the cotton had been off of the bed for some time and after the plants had wilted. The rot started at or near the soil level and extended up the stem 1 to 5 inches, the latter height only rarely. The rotted areas were brown to almost black (Fig. 1), sometimes soft, and the stems often were split longitudinally. Often, the basal leaves were partially to completely rotted. This appeared to be the most usual avenue of entry to the stem. Microscopic examination of affected plants showed the presence of abundant, actively motile, bacteria.

Affected plants, some nearly completely girdled, were set in the field so that the rotted area and some healthy stem tissue came below the soil. All these plants recovered and grew normally. Discolored areas were found

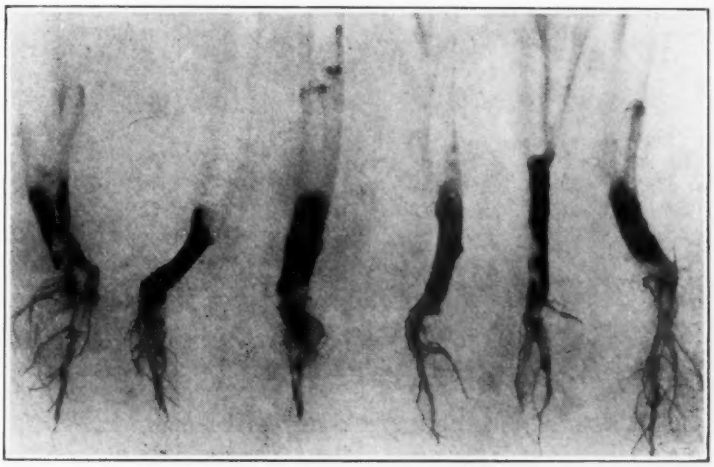

FIG. 1. Tobacco seedlings, transplanting stage, affected with blackleg. Natural infection.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

where healing over occurred, but attempts to isolate bacteria from such areas were not successful.

Bits of affected tissue from seedling plants were removed aseptically, crushed in melted potato-dextrose agar, and plates poured. In from 14 to 20 hours convex, glistening, smooth to amoeboid, translucent, colonies of bacteria developed. Turkish tobacco plants, 8 to 10 inches tall, were inoculated with 48-hour-old potato-dextrose agar tube cultures, from representative colonies, by means of needle stabs half way up the stems. Following inoculation, the plants were placed in moist chambers. Twelve hours after inoculation, water-soaked discolored areas were present around the needle pricks, and in 36 hours the rotting had extended along the stem 2 to 3 inches, above and below the stab. The leaves in the neighborhood of the rotted area drooped, and in some plants the rot extended into the petioles (Fig. 2, A). When removed from the bell jars, some plants broke at the rotted areas. Bacteria reisolated from these plants again caused typical stem rot in Turkish tobacco plants.

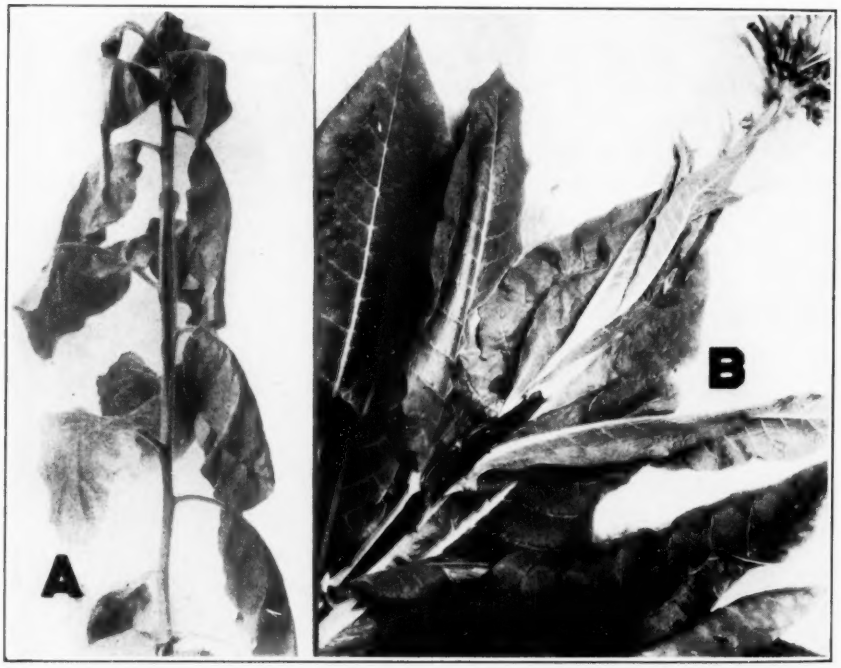

FIG. 2. A. Turkish tobacco plant inoculated with tobacco-blackleg organism. Longitudinal split, drooping leaves, and hollow stalk are shown. B. White Burley tobacco plant inoculated with tobacco-blackleg organism. Bacteria were inserted into a pin prick into the pith 8 inches below the top. Photographed 3 days after inoculation.

Forty-eight-hour-old cultures of the tobacco organism were used to inoculate almost mature White Burley plants in the field. The bacteria were inserted into the pith 8 inches below the tip, near the middle of the stem, and 6 inches from the base of the plants. The stabbed areas were wrapped with moist cotton and paper. After 20 hours there was a brownish, soft, rotted area extending up and down the stem 1½ inches on either side of the stab in the plant inoculated near the top. After 24 hours more the rotted area was 7 inches long and almost girdled the stem. The plants at this time and later were typical of those affected with the disease known as hollow stalk (Fig. 2, B). In the plants inoculated near the middle of the stem and near the soil, the lesions were never longer than 3 inches, and, after 2 weeks, wound tissue formed around these areas. White Burley tobacco plants in the same planting were topped, bacteria pricked into the exposed pith, and moist cotton placed around the inoculated areas. A brown, soft, rotted, area 3 inches long developed in 20 hours. The pith at the top was a soft, slimy, brownish mass with an unpleasant odor. The pith rot seldom extended more than ½ inch down the stem, in contrast with the more extensive rot, when the outer tissues were also infected.

COMPARATIVE STUDIES OF THE TOBACCO ORGANISM WITH OTHER SOFT-ROT ORGANISMS

It was suspected that the organism might belong to the soft-rot group. Through the kindness of J. G. Leach, of the University of Minnesota, three cultures of *Bacillus carotovorus* Jones (I, C, and 3–A) and one of *B. aroideae* Townsend were obtained.[2] Limited comparative cultural, physiological, and pathogenicity studies were made with these and the tobacco organism. All of them hydrolyzed starch, were gram-negative aërobes, and produced filiform, convex, glistening, smooth, and translucent colonies on beef-peptone-agar slants. *Bacillus aroideae* and the tobacco organism clouded beef-peptone broth more than the cultures of *B. carotovorus*. *Bacillus aroideae* and the tobacco organism produced only acid in beef broth containing 1 per cent glucose, sucrose, and lactose, whereas the cultures of *B. carotovorus* produced both acid and gas.

Comparative pathogenicity on potatoes, carrots, and tobacco. The cut ends of carrots, surface-sterilized with 1 to 1,000 bichloride of mercury, were stabbed with a needle dipped into 48-hour-old broth or agar cultures of *Bacillus carotovorus*, *B. aroideae*, and the tobacco organism. After inoculation the carrots were placed in a damp chamber. *Bacillus aroideae*, the tobacco organism, and one culture of *B. carotovorus* (C) produced soft

[2] The writers wish to thank Dr. Leach for helpful suggestions and the Division of Plant Pathology and Botany of the University of Minnesota, where some of this work was done, for laboratory facilities.

rot of the carrot. Carrots, rotted with *B. aroideae*, and the tobacco organism were similar and had a brown discoloration not present in those affected with *B. carotovorus* (Fig. 3).

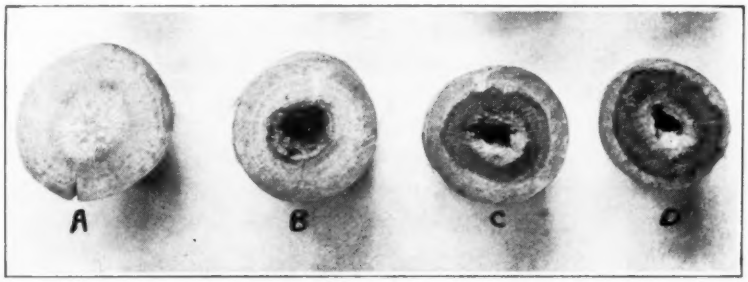

FIG. 3. Cut ends of carrots inoculated with soft-rot bacteria and the tobacco-blackleg organisms. A. Check. Not inoculated. B. *Bacillus carotovorus* (C). C. *B. aroideae*. D. Tobacco-blackleg organism.

Half-inch cylinders were cut from surface-sterilized potato tubers by means of a flamed cork borer. Inoculum from the cultures used to inoculate the carrots was placed on the cut surfaces of the cylinders near the middle, and these were replaced in the holes in the tubers. The latter were placed in a damp chamber. All the cultures of *Bacillus carotovorus* produced some soft rot after 24 hours. Two (I and 3-A) produced only a slight soft rot that did not spread after 48 hours, while the culture (C)

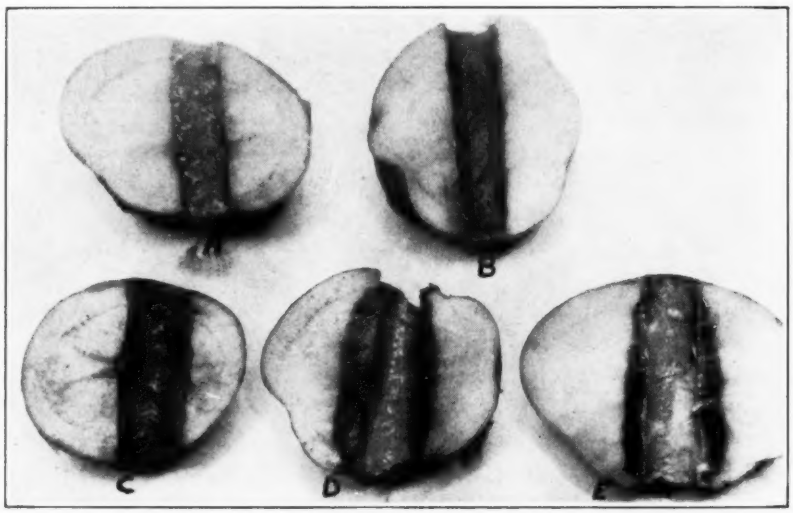

FIG. 4. Potatoes inoculated with soft-rot bacteria and the tobacco-blackleg organism. A. Check. Not inoculated. B. *Bacillus carotovorus* (3a). C. *B. carotovorus* (C). D. *B. aroideae*. E. Tobacco-blackleg organism.

that rotted carrots caused a soft rot extending ⅛ inch into the plug and the same depth into the tuber around the plug. After 24 hours the plugs of tubers inoculated with *Bacillus aroideae* and the tobacco organisms were soft, slimy, masses; and, 24 hours later, the rot had extended into the tuber ½ inch around the plug (Fig. 4).

Two cultures of *Bacillus carotovorus* (C and 3-A), *B. aroideae*, and the tobacco organism produced stem rot of 6-inch Turkish tobacco plants, like that previously described for the tobacco organism.

IDENTITY OF TOBACCO ORGANISM

The tobacco organism resembles *Bacillus aroideae* morphologically, culturally, physiologically, and pathogenetically. Both are peritrichous rods, 2.5 to 3 μ in length by 1 to 2 μ in diameter (Fig. 5). Both react the same on sugar media and differ from *B. carotovorus* in the production of acid but not gas in dextrose, lactose, and sucrose broths. Both produce the same type and degree of rotting on tobacco plants, potato tubers, and carrots. The tobacco organism seems to be one of the soft rot organisms and is probably the same as *B. aroideae* which Massey[3] considers as a separate species from *B. carotovorus*.

DISCUSSION

Bacteria of the soft rot group have been suggested as a cause of hollow stalk of tobacco in the field, but, so far as the writers are aware, injury from these organisms has not been reported in plant beds. The similarity

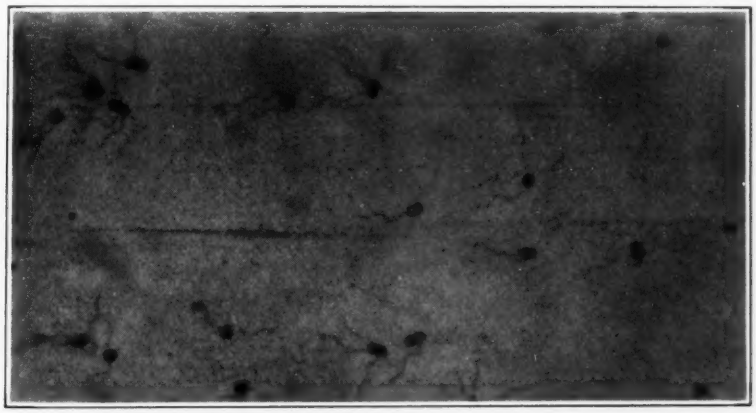

FIG. 5. Tobacco-blackleg organism. Forty-eight-hour-old agar culture. Stained by Casares-Gils method. ×1,000.

[3] Massey, A. B. A study of *Bacillus aroideae* Townsend the cause of a soft rot of tomato, and *B. carotovorus* Jones. Phytopath. **14**: 460–477. 1924.

of symptoms of tobacco plants affected with *Bacillus carotovorus* and *B. aroideae* suggests that either of these may be pathogenic to tobacco and, with favorable conditions, might cause blackleg of seedlings, or hollow stalk of older plants in the field.

The writers observed this disease in a plant bed on the Experiment Station farm at Lexington a few years ago. Discussions with growers indicate that they are familiar with the disease and that they fear it during rainy periods when the plants are large and crowded in the bed.

Leach[4] has demonstrated that the pathogene of potato blackleg, which, in a later paper,[5] he assigns to the soft rot group, can overwinter in the soil in Minnesota. It is probable that soft rot bacteria may inhabit many soils. If temperature and moisture conditions be favorable, soft rot bacteria may enter seedlings through the hydathodes, as it is not unusual to find the rot starting at the tips of leaves that touch the soil. The organism might enter the tobacco plant through insect wounds, but no case of this kind has been observed by the writers. The mode of entry into plants in the field is not known.

SUMMARY

A stem rot of tobacco seedlings at the transplanting stage is described as it was observed in two plant beds in Kentucky. Microscopic examination of affected plants showed the presence of actively motile bacteria. The name, blackleg, is suggested for the disease because of its resemblance to blackleg of potato.

Bacteria isolated from affected plants caused a stem rot of Turkish tobacco in the greenhouse and a stem and pith rot of Burley tobacco in the field. Soft rot of potatoes and carrots was produced by cultures of the stem rot pathogene.

Comparative studies indicate that the stem rot organism is similar to, if not identical with, *Bacillus aroideae* in that it produces acid but not gas on beef broth containing lactose, dextrose, and sucrose.

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[4] Leach, J. G. Potato blackleg: The survival of the pathogene in the soil and some factors influencing infection. Phytopath. 20: 215–228. 1930.

[5] Leach, J. G. The identity of the potato blackleg pathogene. Phytopath. 20: 743–751. 1930.

SEED TRANSMISSION OF COWPEA FUSARIUM WILT

JAMES B. KENDRICK

The wilt of cowpeas (*Vigna sinensis* (L.) Endl.) caused by *Fusarium tracheiphilum* (E.F.Sm.) Wr., is a serious factor in nearly all sections of California where blackeye cowpeas are grown. In many cases, the soil has become so thoroughly infested with the wilt that the growing of blackeye cowpeas is no longer profitable. The increasing severity of the disease has forced many growers to use new areas for growing "blackeyes," as they are commonly known in California. Many complaints have been received of the occurrence of wilt in areas where blackeyes have not been previously grown. Observational evidence pointed to the possibility that the disease was being spread with the seed. In so far as the writer is aware, there is no record to show that this disease is seed-borne. Since the wilt is of major importance in the growing of blackeye cowpeas in California, it seemed advisable to determine experimentally if the agent responsible for the disease was being disseminated with the seed.

The disease was first described by Smith[1] in 1899. In 1902 Orton[2] published a rather comprehensive account of the same disease. As was pointed out by Orton and from three years' observations in California, cowpea wilt does not appear until the plants are about 6 weeks old. At first, a few plants are noticed throughout the field with pale green, flaccid leaves, which soon turn yellow and drop from the plant. The plants showing the disease early in the season usually die prematurely and fail to mature seed. As the season advances, more and more plants show the disease, as evidenced by their dwarfed condition, yellowness, and, in many cases, death of the infected plants. However, not all diseased plants show external symptoms. In experimental plots, where each plant has been pulled at the end of the season and the stem cut for evidence of the disease, many large, apparently healthy plants have been found with a greatly swollen and roughened condition of the lower part of the stem and main root (Fig. 1, A). When the stems of such plants are examined more carefully, the vascular system shows as a dark-brown mass of disintegrated tissue with only the outer cortical area showing evidence of life. The vascular discoloration often extends throughout the plant. Mature plants have been observed at the end of the season, which appeared vigorous and healthy, but, on examination of the root system, they were found to be in a badly dis-

[1] Smith, Erwin F. Wilt disease of cotton, watermelon and cowpea. U. S. Dept. Agr., Div. Veg. Phys. and Path. Bul. 17. 1899.

[2] Orton, W. A. Some diseases of the cowpea. U. S. Dept. Agr., Bul. Plant Indus. Bul. 17. 1902.

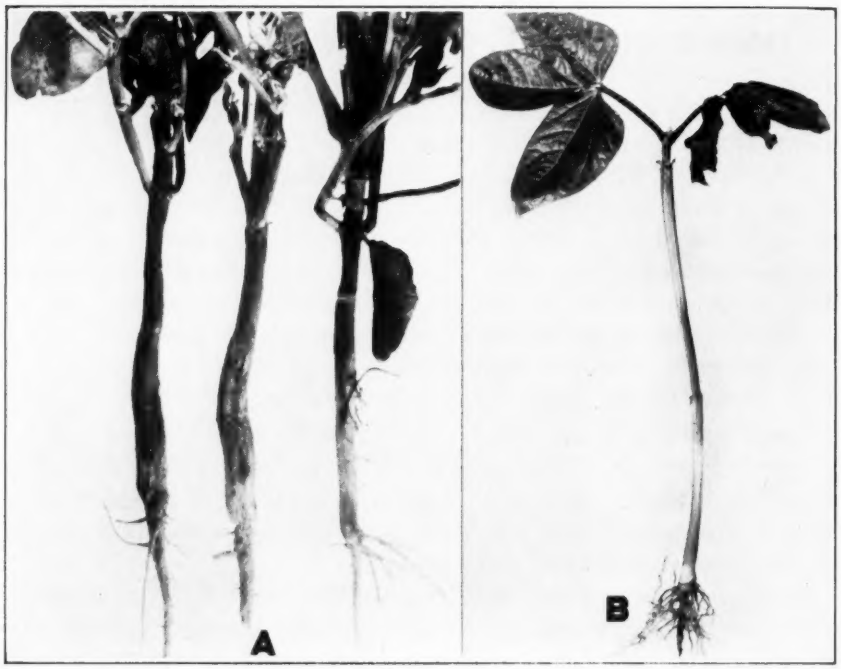

FIG. 1. A. Two blackeye-cowpea plants on the left, severely infected with *Fusarium tracheiphilum*, showing the swollen and roughened condition of the lower stem and main root, while the foliage showed no evidence of disease. Such plants often mature a fair amount of seed and are harvested with the healthy plants. Healthy plant on the right. B. Seedling blackeye cowpea grown in steam-sterilized soil from seed harvested by heating mature diseased plants in a burlap bag. The wilted leaf on one side denotes the first evidence of wilt.

eased condition. Other plants, with a much less severely diseased vascular system, die from the disease.

Many plants that show the disease by midsummer also survive and mature a partial crop of seed even though they lose all or part of their leaves and show severe vascular infection throughout the plant. Since many diseased plants mature seed, the possibility that the fungus responsible for the disease might be carried with the seed seemed likely.

In order to determine whether the fungus penetrated the seed and was harbored as intraseminal mycelium, a series of cultures were made. Macroscopic examination of diseased plants showed the brown discoloration in the vascular system of all parts of the plant. Peduncles with attached ripening pods were removed from severely diseased plants. The pods were carefully plucked and the peduncles surface-sterilized with mercuric chloride 1–1000 and then washed in sterile water. Small cross sections

were cut aseptically from the basal and apical end of each peduncle and planted on potato-dextrose agar. The pods previously removed from the peduncles were carefully opened with sterile tweezers and the first pea from the basal end of each pod was plated on potato-dextrose agar, care being taken to place the hilum in contact with the agar.

Platings were made from the basal end of 205 peduncles, of which 34.6 per cent showed the presence of *Fusarium tracheiphilum*. Out of 271 apical ends of peduncles thus cultured, 4.8 per cent yielded *F. tracheiphilum*, while 315 seeds plated as stated above failed to show any evidence of *F. tracheiphilum*. The pathogenicity of the cultures was determined by greenhouse inoculations in sterile soil. The above results indicate that the causal fungus quite often penetrates the peduncle and in some cases reaches the pod, but in no case was there evidence of penetration of the first seed in the pod. However, more extensive tests may show that the fungus does, in some cases, enter the seed.

Two methods of securing seed for testing were used. In one instance, an attempt was made to simulate harvest conditions as near as possible and, in the other case, ripe pods were carefully removed from severely diseased plants by hand. It may be well to state that the general method used in harvesting beans in California is to cut the plants just beneath the soil by running a cutter along the row. The plants are then raked into windrows and allowed to dry, after which they are run through a threshing machine. Since a great deal of diseased material is thus run through the threshing machine, no doubt abundant spore material is liberated and lodges on the seed coat. While harvesting some experimental plots in 1929, a large number of diseased plants were pulled and placed in a large burlap bag. After the plants were dry, the beans were threshed by beating the plants in the bag and the seed removed from the trash by means of an electric fan. At the same time a quantity of seed was harvested by hand-picking ripe pods from a number of plants showing severe wilt. The 2 lots of seed were kept separate and used for subsequent greenhouse tests. For convenience the 2 lots of seed were designated as threshed seed and hand-picked seed and will be thus referred to in this paper. All seed tests were made in soil that had been steam-sterilized at 40-pounds pressure for 3 hours.

On October 24, 1929, 4 flats were planted with threshed seed and 20 6-inch pots with hand-picked seed. The first evidence of wilt occurred 43 days after planting. As soon as a plant showed evidence of disease (Fig. 1, B), it was removed and cultures made. Final notes were taken on January 21, at which time all plants were removed and the main stem cut for evidence of vascular infection. Many plants showed symptoms more or

less typical of those occurring under field conditions. The results of the above test showed that out of 152 plants grown from threshed seed 40, or 26.3 per cent, developed wilt symptoms, while 147 plants grown from hand-picked seed remained healthy.

Fourteen flats of sterile soil were planted on January 31, 1930, with the same lot of threshed seed used in the previous trial. In this test, 1,028 plants developed, of which 168, or 16.3 per cent, developed wilt.

Since there was a possibility of secondary spread from the early diseased plants to those in close proximity in the two former trials, a third set of plantings was made on April 10, 1930, in which single peas were planted in 4-inch pots of soil. At the same time, 10 peas were planted in each of 31 6-inch pots and 188 6-inch pots were planted with 3 lots of hand-picked seed. The results are presented in tabular form below.

TABLE 1.—*A comparison of seed transmission of cowpea Fusarium wilt where the seeds were harvested by two different methods and grown under different conditions*

Seed lot	Method of growing	Number of plants	Percentage showing wilt
Threshed seed	10 peas in each 6-inch pot	259	8.5
"	1 " 4- "	574	3.9
Hand-picked seed Lot 1	10 " 6- "	210	0
" " 2	10 " 6- "	344	0
" " 3	10 " 6- "	255	3.5

The results presented in table 1 indicate that there may have been some secondary spread, where more than 1 plant was grown in a container. However, since 214 young plants growing in individual 4-inch pots were killed by small root maggots, it is possible that the incidence of the disease may have been increased if these plants had not been destroyed. It will also be noted that in 1 lot of hand-picked seed 3.5 per cent of wilt developed.

In the fall of 1930 2 additional seed lots were collected, using the same methods of harvesting that were used in 1929. On November 5, 1930, 12 flats were planted with 1930-threshed seed and 10 flats with 1930-hand-picked seed. The results showed that of the 574 plants grown from threshed seed 65, or 11.3 per cent, developed wilt, while the 560 plants from hand-picked seed showed 1 with definite wilt symptoms.

The evidence proves quite conclusively that the agent responsible for cowpea wilt is carried on the seed. In 2 out of 5 trials, a small percentage of wilt occurred where plants were grown from hand-picked seed. As to whether this resulted from the organism being inside the seed coat or from

other sources, positive proof is lacking. This point is being investigated further.

In order to determine if the causal agent of cowpea wilt lives from one season to another on the seed, 6 flats of sterile soil were planted on September 12, 1930, with 1-year-old threshed seed. The results showed that out of the 305 plants 22, or 7.2 per cent, showed wilt symptoms. A summary of the seed tests previously made with the same lot of seed shows an apparent reduction in the percentage of wilt as the seed becomes older. Seed 43-days old showed 26.3 per cent wilt; 142-days old, 16.3 per cent wilt; 205-days old, 8.5 per cent wilt; and 1-year old, 7.2 per cent wilt.

Additional evidence that the seed is responsible for the dissemination of cowpea wilt was secured when a commercial lot of seed, suspected of harboring wilt, was tested. A grower used 2 lots of seed to make his 1930 planting. The planting from 1 of these seed lots developed a small percentage of wilt, and circumstances pointed to the seed as the source of the disease. A subsequent greenhouse test in which 320 plants were grown from this seed showed 4, or 1.25 per cent, showing definite wilt symptoms.

SUMMARY

Preliminary trials have failed to show that the fungus (*Fusarium tracheiphilum*), causing cowpea wilt, penetrates the seed. Evidence is presented that the fungus is transmitted on the seed where machine methods are used for harvesting. Hand-picked seed from diseased plants showed a low percentage of wilt in 2 out of 5 trials. It has also been found that a rather high percentage of wilt resulted from using seed 1 year old.

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SEED-TREATMENT AND DATE-OF-SOWING EXPERIMENTS WITH SIX VARIETIES OF FLAX[1]

L. C. Burnett[2] and Chas. S. Reddy[3]

Flaxseed production in Iowa was greater in 1930 than in any year since 1908. There was an increase of 75 per cent in acreage and 110 per cent in production over the 10-year average (1920–1929). In view of this added interest, preliminary experiments were inaugurated to study the influence of date of sowing and of seed treatment upon the stand and yield of flax. These experiments were conducted with the leading wilt-resistant varieties at four places in Iowa.

Yields of 6 varieties are presented in table 1 for the May 1 and May 14 dates of sowing. The sowing on May 31 produced such a poor crop that it was not harvested but was discarded in the field.

TABLE 1.—*Effect of date of sowing on acre yields of 6 varieties of flax at the Agronomy Farm, Ames, Iowa, 1930*

	Bison cheek	N D R 114	Red-wing	Lin-ota	Buda	Bison	Rio	Mean
Sown May 1								
Acre yield (bu.)	17.72	15.20	16.82	14.55	12.52	18.05	14.27	15.59
Sown May 14								
Acre yield (bu.)	7.92	9.22	12.90	8.10	5.45	7.55	7.12	8.32
Loss from late sowing								
Bushels	9.80	5.98	3.92	6.45	7.07	10.50	7.15	7.29
Percentage	55.3	39.6	23.3	44.4	56.5	58.2	50.2	46.6

The data in table 1 show that satisfactory yields were obtained from the first sowing; little more than half as much (53.4 per cent) from the second sowing; and practically no yield from the third. The differences, no doubt, were accentuated by the exceptionally dry season of 1930.

Thirty observations at Ames on stands and yields showed variations from 119 to 506 plants per 17-ft. row with corresponding yields ranging from 130 to 197 gm. (13.0 to 19.7 bu. per acre). In these data an increase in yield of 1 bushel per acre was obtained with each increase of 1 plant, from 8 to 10 plants per square foot. Above that number (10 per square foot) an increase of about 4 plants per square foot was required to secure an increase of one bushel per acre.

[1] Published with the approval of the Director as a Journal Paper of the Iowa Agricultural Experiment Station, Ames, Iowa.

[2] Chief in Cereal Breeding, Farm Crops Section.

[3] Assistant Chief, Botany and Plant Pathology Section.

Seed-treatment experiments, using seed of Bison flax (96 per cent germination) obtained from Fargo, North Dakota, were also conducted on the Agronomy Farm at Ames (10 replications sown on May 1), on Plant Pathology plots at Ames (10 replications sown on April 28), at Belmond (3 replications sown on April 22), and at Mason City (3 replications sown on April 23). The rate of sowing was 5 gm. or approximately 940 seeds per 17-ft. row. Dust fungicides comprising 2 trial dusts (G-2-2 and 31C), Corona Oat Dust (COD), Ceresan (Cer), and American cyanamid No. 7 (Am. Cy. 7) were used at the rate of 2 ounces per bushel. The stand data were obtained when the plants were mature by pulling, and counting 5 of the replications at the Agronomy Farm, 1 at the Plant Pathology plots, 3 at Belmond, and 3 at Mason City (table 2).

TABLE 2.—*Field stands from nontreated and treated Bison flaxseed sown at four places in Iowa, 1930*

Location of experimental field	Replications	Number of plants per 17-ft. row[a]					
		Non tr. Check	Treated with:				
			G–2–2	31–C	COD	Cer.	Am. Cy. 7
Ames, Agr. Farm	5	140	271	185	190	401	169
Ames, Path. Plots	1	362	481	455	398	516	390
Belmond	3	502	462	491	451	598	462
Mason City	3	277	449	412	351	406	252
Weighted mean	(12)	283.2	380.6	340.8	312.8	461.1	281.2
Percentage of check							
Ames, Agr. Farm	5	100	193.5	132.1	135.7	286.3	120.7
Ames, Path. Plots	1	100	132.8	125.2	110.0	142.5	107.7
Belmond	3	100	92.1	97.8	89.8	119.2	92.1
Mason City	3	100	162.0	148.7	126.7	146.5	91.0
Weighted mean	(12)	100.0	134.2	120.2	110.3	162.7	99.3

[a] Approximately 940 seeds were planted in each row.

The data in table 2 show that increases in stand were obtained from all the treatments except Am. Cy. 7. Nontreated seed produced percentage stands ranging from 15 to 53 (140 to 502 plants per row). Under the same conditions and with seed of the same lot the percentage stands from the best seed treatment, Ceresan, ranged only from 42 to 63 (401 to 598 plants per row).

The yield data from the seed-treatment plots are presented in table 3, and data from tables 2 and are summarized in table 4.

TABLE 3.—*Yields from nontreated and treated Bison flaxseed sown at four places in Iowa, 1930*

Location of experimental field	No. Replications	Yield in grams per 17-ft. row					
		Non tr. Check	Treated with:				
			G–2–2	31–C	COD	Cer.	Am. Cy. 7
Ames, Agr. Farm	5	145	168	147	148	194	169
Ames, Path. Plots	1	140	200	120	140	195	110
Belmond	3	163	160	162	157	187	149
Mason City	3	183	187	202	188	223	181
Weighted mean	(12)	150.2	173.8	162.2	159.6	199.6	162.1
Percentage of check							
Ames, Agr. Farm	5	100	115.8	101.4	102.0	133.7	116.5
Ames, Path. Plots	1	100	142.9	95.7	100.0	139.2	78.6
Belmond	3	100	98.2	99.4	96.4	114.6	91.4
Mason City	3	100	102.1	110.4	102.7	121.8	99.0
Weighted mean	(12)	100.0	115.6	108.0	106.3	132.9	107.9

TABLE 4.—*Relation of stand to yield in the 12 replications of the seed-treatment experiment conducted on four experimental fields in Iowa in 1930*[a]

Seed treatments	(None) Check	G–2–2	31–C	COD	Cer.	Am. Cy. 7
Stand:						
Plants per 17-ft. row	283.2	380.6	340.8	312.8	461.1	281.2
Percentage of check	100.0	134.6	120.3	110.4	162.7	99.3
Yield:						
Grams per 17-ft. row	150.2	173.8	162.2	159.6	199.6	162.1
Percentage of check	100.0	115.6	108.0	106.3	132.9	107.9

[a] Data from tables 2 and 3.

Table 4 shows (1) that the highest field stand produced the highest yield and that, except for Am. Cy. 7, the yields are directly correlated with the stands; and (2) that all the treatments, except Am. Cy. 7, gave increases in stand and that all the treatments gave increases in yield.

The yield data presented in table 5 for 26 replications include 14 for which no stand counts were made.

Table 5 shows that yields were increased by both G-2-2 and 31-C, but better gains were made following the use of Ceresan.

TABLE 5.—*Acre yields[a] from nontreated and treated Bison flaxseed sown at four places in Iowa, 1930*

Location of experimental field	Replications	Nontreated Check	Treated with: G–2–2	31–C	COD	Cer.	Am. Cy. 7
Ames, Agr. Farm	10	15.15	17.95	16.30	13.40	19.75	13.90
Ames, Path. Plots	10	11.55	12.45	12.20	11.20	14.85	11.20
Belmond	3	16.30	16.00	16.20	15.70	18.70	14.90
Mason City	3	18.30	18.70	20.20	18.80	22.30	18.10
Weighted mean	(26)	14.26	15.70	15.16	13.44	18.04	13.46
Percentage check		100.0	110.1	106.30	94.20	126.50	94.4

[a] Calculated from replications of 17-ft. rows.

Ceresan also improved the stands and yields of 5 other varieties in plots of 2 replications each sown on May 14. The effect on stand and yield is presented in table 6, in addition to the data for Bison, which has been brought forward from the foregoing tables.

TABLE 6.—*Effect of Ceresan seed treatment on stands and yields of 6 varieties of flax. Iowa, 1930*

Variety	Replications	Stand: Plants per 17′ Nontr.	Stand: Plants per 17′ Tr.	Tr. in per cent of nontr.	Acre yield: Nontr.	Acre yield: Tr.	Bushels increase	Tr. in per cent of Nontr.
Bison	26				14.26	18.04	3.78	126.5
Bison	12	283.2	461.7	163.0	15.02	19.96	4.94	132.9
NDR-114	2	598	808	135.2	7.75	9.75	2.00	125.8
Redwing	2	456	671	114.7	11.75	13.25	1.50	112.6
Linota	2	359	819	228.0	7.00	9.00	2.00	128.5
Buda	2	252	445	176.4	4.50	6.50	2.00	144.5
Bison	2	223	331	148.1	6.50	9.00	2.50	138.5
Rio	2	124	247	199.0	6.75	8.25	1.50	122.1
Mean of six varieties	(12)	335.3	553.5	165.1	7.375	9.291	1.917	126.0

Table 6 shows that the stand and yield of every variety used were improved and that the mean increase for all the varieties was 65 per cent in stand and 26 per cent in yield.

SUMMARY

Due to the exceptionally dry season, satisfactory yields of flax were obtained only from the first sowing, May 1; little more than half as much (53.4 per cent) from the second sowing, May 14; and practically no yield from the third sowing, May 31.

When Bison flax of high vitality was grown under a wide range of conditions the percentage of seed which produced plants varied from 14.9 to 53.4. In the same experiments seed of the same lot treated with Ceresan gave percentage stands of 42.6 to 63.6.

Seed treatment with Ceresan increased the yields of Bison flax 26.5 per cent in 26 replications and the yields of 5 other varieties (2 replications each) by percentages varying from 12.6 to 44.5.

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PYTHIUM BUTLERI—THE CAUSE OF A BEAN WILT

L. L. HARTER AND W. J. ZAUMEYER[1]

INTRODUCTION

In a recent publication Harter and Zaumeyer[2] reported a wilt of beans, *Phaseolus vulgaris*, that occurred almost simultaneously in 1930 at Greeley, Colo., and Rosslyn, Va.

Not many days after the disease was observed in Colorado, one of the local growers reported that stem girdle, caused by *Bacterium phaseoli* E.F.S. or *Bact. medicaginis* var. *phaseolicola* Burk., was present in a number of bean fields and was causing the death of many plants. In examining these fields no bacterial blight was found. The death of the plants in this instance was caused by a Pythium which produced symptoms similar to those observed in other fields in the same general locality.

Pythium has long been known to cause bean-root rots and damping off of seedlings. However, this is the first time the writers have observed this organism to cause serious losses of almost mature plants under field conditions.

The purpose of this paper is to supplement the previous report of the disease and to give a more detailed account of the malady as it appeared under field conditions, with special reference to symptoms and conditions that favor its spread and development.

DESCRIPTION OF THE DISEASE

Pythium wilt begins as a water-soaked infection of the stem of the plant at about the soil line and progresses upward into the branches and petioles of the lower leaves. If the soil is drawn up to the stem, the initial infection is in the region of the lower branches. The organism progresses rapidly from the point of infection up the stem and into the branches, causing a water-soaked appearance of the surface. While there is no discoloration of the invaded tissue, the cortex is softened so that it can be readily separated from the vascular tissue, a characteristic said to be common to the Pythiums as a group. The infection rarely extends below the surface of the soil or to the roots.

The earliest symptoms of the disease are characterized by a slight wilting of the foliage in the warmer part of the day, followed by a return to the normal turgidity of the leaves at night. A few days later these symptoms are followed by pronounced continuous wilting in which the

[1] Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

[2] Harter, L. L., and W. J. Zaumeyer. A wilt of beans caused by Pythium. (Abst.) Phytopath. **21**: 115. 1931.

branches droop noticeably, followed a few days later by death of the plant. If weather conditions are favorable, the mycelium will grow profusely over the dead cortex, often from the soil line into the branches. Under suitable conditions of temperature and humidity, the disease progresses so rapidly that the plant may be killed in a few days after infection.

HISTORY AND GEOGRAPHIC DISTRIBUTION

It is not believed that this disease has been observed previously on bean plants except as a damping off of seedlings and as a root rot. The distribution was not general in Colorado in 1930, but the disease was noted in one particular section where it was found in five large plantings, in some cases causing a reduction in stand of from 10 to 12 per cent. In most fields the amount was less. It was observed at Rosslyn, Va., for the first time in 1930.

SUSCEPTIBLE VARIETIES

It is believed that all snap-bean varieties are susceptible to the disease; however, it was observed under field conditions only on the Late Stringless Green Refugee, Round Pod Kidney Wax, Improved Kidney Wax, Hodson Wax, Giant Stringless Green Pod, and Black Valentine.

CONDITIONS FAVORABLE FOR INFECTION AND GROWTH OF THE FUNGUS

Pythium wilt of beans was observed in Colorado and at Rosslyn, Va., about July 15, 1930. In some respects the disease appeared under quite different climatic conditions and in other respects under similar ones. In Virginia no rain had fallen for several weeks. The soil, a light sandy loam, in which the beans were growing, was very dry and a dust mulch 3 or 4 inches deep covered the surface. The temperatures were extremely high when the disease was at its worst, ranging in the daytime from 32° C. to 41° C.

In Colorado the disease was observed on somewhat heavier soil. The water there is supplied mostly by surface irrigation, although rains may occur during the summer months. However, up to the time when the disease was noted, only 0.15 of an inch of rain had fallen. The season was very dry and the beans, which were from 10 to 12 inches high and growing vigorously, had been irrigated at least twice. The disease was observed in several fields close to one another and it was especially severe in a portion of one field that had been flooded by irrigation water. The results from this field alone would indicate that there is a direct correlation between wet soil and Pythium wilt. On the other hand, the disease occurred, with identical symptoms, in other fields nearby that had not been flooded with irrigation water.

Since the temperature was high in both Colorado and Virginia, the experimental data and field observations indicate that it was more likely to be the determining factor in the severity of the disease than soil moisture. It is generally believed that high relative humidity, and high moisture content of the soil are essential to the incidence of the diseases caused by Pythium.

Notwithstanding the fact that a drought prevailed in Virginia when wilt was prevalent, records taken by a hygrothermograph showed that the relative humidity during the night was close to saturation, a condition which would be favorable to infection by Pythium and its subsequent development. In irrigated fields of the arid regions of the West, a high relative humidity or nearly saturated atmosphere might and probably did occur at the crown of plants for several hours at a time. As a matter of fact, hygrothermograph records taken in irrigated bean fields of the West showed that, as in the East, the relative humidity during certain nights was quite high, although it did not extend over so long a period of time. The conclusion to be drawn from these data is that the relative humidity is sufficiently high for a long enough period of time in both the East and West to permit infection.

Pythium butleri, the cause of wilt of beans, is widely distributed. It occurs on the roots of many crops and is the cause of decay of beans in shipment. Harter and Whitney[3] showed that *P. aphanidermatum* causes a nesting and decay of beans in transit from the field in Florida to the northern markets. It has also been found on the fibrous roots of beans, and to be a cause of damping off of seedlings. *P. aphanidermatum* is now found to be *P. butleri,* the form that occurs on the roots of beans, and is the cause of nesting.

INOCULATION EXPERIMENTS

Cultures were made from diseased plants from both Colorado and Virginia, and the organisms from the two localities were identical. Inoculation experiments were made on seedlings and young plants beyond the seedling stage, by inserting mycelium into wounds made at the cotyledonary node. Some of the plants were covered with bell jars or inclosed in infection chambers to maintain a high humidity, while others were left uncovered. The percentage of infection was sufficiently high to demonstrate the causal relation of the fungus and reisolation proved the identity of the organism. The symptoms were typical of the disease as observed in the field under natural conditions. The checks remained healthy. Wilting of inoculated plants did not occur at ordinary greenhouse temperature, which

[3] Harter, L. L., and Whitney, W. A. A transit disease of snap beans caused by *Pythium aphanidermatum.* Jour. Agr. Res. 34: 443–447. 1927.

was maintained at about 21° C. during the daytime and about 16° C. at night. When these plants were placed in an infection chamber at 30° C. and in an almost saturated atmosphere, wilting occurred in three to four days.

It is believed that temperature is a more important factor than humidity in the occurrence of Pythium wilt. Evidence to that effect was obtained from a greenhouse experiment in which two sets of plants, each consisting of nine plants, were similarly inoculated by inserting the fungus into the stem at the soil level. Sphagnum was placed around the plants at the points of inoculation and kept moist in the one series, while the other series remained uncovered. Both sets, including the checks, were held at ordinary greenhouse temperatures and no infection resulted. When similar plants were placed in infection chambers at a temperature of 36° C., wilting followed in three to four days. It appears that a combination of high temperature and humidity provides ideal conditions for infection but that high temperatures are more essential for infection than high humidities.

REPORT OF THE FIFTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

OFFICERS:

President.....E. Carsner, U. S. Department of Agriculture, Riverside, California.
Vice-President.....J. M. Raeder, University of Idaho, Moscow, Idaho.
Secretary-Treasurer.....B. A. Rudolph, U. C. Deciduous Fruit Station, San Jose, California.
Councilor.....C. E. Owens, State College, Corvallis, Oregon.

The meetings of the Pacific Division of the American Phytopathological Society were held in conjunction with those of the first national summer meetings of the American Association for the Advancement of Science and Affiliated Societies, at the California Institute of Technology at Pasadena, California, on June 16, 1931.

Thirty-five members, with friends and visitors, were in attendance. Twelve papers were read, the titles and abstracts of which are included in the present report.

The meetings this year were designed to permit of a great number of interesting excursions, many of which were enjoyed by visiting members. On Monday, June 15, the members of the American Association for the Advancement of Science and Affiliated Societies were accorded a reception and private showing of the magnificent Huntington Gardens at San Marino, a suburb of Pasadena. On this same occasion the famous library and art gallery also were thrown open to members of the Association. An orchestra played out of doors under the great oaks, which are one of the fine features of the gardens, and refreshments were served throughout the afternoon.

On Friday, June 19, the Pacific Division pathologists met with Section G of the American Association for the Advancement of Science and Affiliated Societies at the Rancho Santa Ana Botanic Gardens as the guests of Mrs. Susanna Bixby Bryant, its founder. Papers pertinent to botanic gardens were read by Douglas H. Campbell, Walter T. Swingle, and H. J. Webber. Following a delicious luncheon a visit was made to the botanic gardens on the estate.

Many other free and interesting excursions to the beaches, moving-picture studios, the Mount Wilson Observatory, etc., were made possible through the courtesy of the Los Angeles Chamber of Commerce and other organizations.

B. A. RUDOLPH, *Secretary-Treasurer*

ABSTRACTS

The curing of exanthema by the injection of copper sulphate into the tree.—H. E. THOMAS.

Results obtained in central California over a period of 2½ years indicate that powdered copper sulphate, injected during the dormant season into holes bored in the base of the tree trunk, may successfully control exanthema in deciduous fruit trees. The amount necessary to cure the disease appears to vary. Two grams was insufficient at times to eliminate all burning in large trees. Injections of 15 and 20 gm. did not cause injury. While the addition of copper sulphate to the soil around the base of diseased trees in one orchard failed to decrease noticeably the amount of burning in the top, the injection of from 4 to 6 gm. of the sulphate into the crown produced trees entirely free of burn or dying tips. Limited trials indicate copper acetate and chloride effectively cure the disease. Copper tartrate, phosphate, and carbonate failed to give satisfactory results.

Speculation arises as to the rôle of copper in this disease. It may be necessary as an essential element for the growth of the tree, diseased trees having insufficient copper; or it may act in neutralizing some toxic substance taken up by the tree from the soil in which the tree is growing.

Developmental stages of Heterodera radicicola in pineapple and cowpea roots.—G. H. GODFREY and JULIETTE OLIVEIRA.

Large numbers of young, actively growing roots of pineapple and cowpea were heavily inoculated with recently hatched larvae of the root-knot nematode and then, at regular intervals, roots were excised, washed, killed in Fleming's, fixed, dehydrated in graded alcohols, and cleared in clove oil. The results were series of roots showing clearly the successive stages of nematode development and relationship to the root tissues, beginning with earliest penetration and ending with the initiation of a new generation. The nematodes were stained dark and stood out clearly in their natural position in the root tissues. A series of photographs of about 16-diameter magnification records this graphically. Permanent slides were made by infiltration with balsam. A striking development from this study is that the length of life history varies greatly between the two host plants, growing conditions being identical, as both plants were inoculated at precisely the same time. The life cycle is much longer in the pineapple roots.

Verticilliosis of strawberries.—H. E. THOMAS.

It appears that *Verticillium albo-atrum* is capable of causing a disease in strawberries. Plants set at San Jose, California, in soil accidentally infected with Verticillium were showing marked symptoms of distress 3 months after planting. The outermost leaves of infected plants wilt, turn brown, and die, or burn at the edge, leaving an irregular green center in the leaf. Affected plants may die slowly but often partially recover. Occasionally, a rather sudden wilt occurs. Verticillium was readily isolated from the crowns of such plants but was never taken from the roots. Several dying plants taken from commercial patches yielded the fungus upon culturing from the crowns. Thirty strawberry plants of the Nick Ohmer variety were set in sterilized soil in 6-inch pots in the greenhouse and inoculated with pure cultures of *Verticillium albo-atrum*. Five months later 50 per cent of the inoculated plants showed wilting and dying in varying degree, similar to that occurring in the field. The remaining 50 per cent appeared to be unaffected by the inoculation. Twenty check plants remained healthy. The fungus was isolated from the crowns of the diseased plants but not from the checks.

A new disease of maize and beans.—W. W. MACKIE.

In the summer of 1929 a new disease was found in maize and beans. The fungus was identified as *Rhizoctonia bataticola* (Taub.) Butler. It is easily recognized by the presence in the stele of dense black sclerotia about 120 to 200 microns in diameter. It is transmitted from sclerotia to sclerotia in maize and beans. In culture media about 6 days are required to again reproduce the sclerotia. In beans and maize the sclerotia are found in the roots and in the plant stems to a point not beyond 10 or 12 inches above the soil. In beans the black sclerotia are found deeply embedded in the stele. In maize and beans they cluster about the vascular bundles but are found also in the pith. Wilt and premature ripening ensue, with a reduction in the size of the plant and the seed. All commercially grown beans in California, except Large Limas, and all subspecies of maize were attacked. Resistant varieties were found.

A preliminary report on resistance to curly top of sugar beets in bean hybrids and varieties.—W. W. MACKIE and KATHERINE ESAU.

Since 1919 curly top of sugar beets has been known to attack beans. To test the resistance of bean hybrids and varieties short rows were infested with infective nymphs of *Eutettix tenellus*, at the fourth-leaf stage, with the following results.

Grades of resistance	1		2		3		4		5	
	Immune, or no visible damage		Slight injury		Considerable injury		Heavy injury		Destruction	
	No. of varieties	Per cent	No. of varieties	Per cent	No. of varieties	Per cent	No. of varieties	Per cent	No. of varieties	Per cent
Phaseolus vulgaris										
White	3	4.7	3	4.7	9	14.0	16	25.0	33	51.6
Pink or rose	5	35.6	2	14.2	2	14.2	1	7.0	4	29.0
Mottled	2	20.0	3	30.0	1	10.0	1	10.0	3	30.0
P. multiflorus										
White			1	100.0						
P. lunatus sieva										
White	0	0.0	1	17.0	4	66.0	1	17.0		

In the cross between Robust, a white pea bean, resistant to mosaic, and California Pink, resistant to curly top, a number of fixed resistant hybrids were secured in both pink and white beans. Correlation between pink or red color and resistance was found to be only partial, indicating that white beans can be secured that are resistant to curly top. Continued resistance to both curly top and bean mosaic may be secured in both white and pink beans of the species *Phaseolus vulgaris*.

Experimental freezing of apple trees.—J. H. CRENSHAW and J. S. COOLEY.

In experimental studies of perennial apple canker it seemed desirable to freeze parts of apple trees having received different preparatory treatments.

Method: The limb to be frozen was fitted into notches in the top of an insulated box containing the refrigerant. Two different refrigerants were used. A mixture of $CaCl_2$ and snow gave a minimum temperature of $-25°$ to $-35°$ F., but required recharging at 3- to 5-hour intervals. By using solid CO_2 in definite amounts as the refrigerant, temperatures could be controlled as low as needed by recharging at intervals of 24 to 36 hours. A temperature of $-40°$ F. was easily obtained. Freezing was done across a fertilizer plot at three different times, viz., late fall, midwinter, and early spring. The types of wounds frozen were healthy calluses, old cankers, and fresh pruning wounds. Some of the calluses had been kept free from aphis, while others were aphis infested.

Results: The killing resulting from freezing on November 30 was much severer than that on January 1. Callus tissue was more susceptible to freezing than normal bark. Woolly-aphis-infested calluses were more susceptible to cold injury than aphis-free

calluses. Fresh wounds were more severely injured than callused wounds. Cankers were more susceptible to injury than sound-healing calluses. No positive correlation between fertilizer treatment and freezing injury was noted.

Tranzschelia punctata on cultivated anemone in the Santa Clara Valley.—C. EMLEN SCOTT and GILBERT L. STOUT.

In March, 1931, attention was called to an anemone planting near San Jose in which a rust infection was severe. Leaves of almond, apricot, cherry, nectarine, peach, plum, and prune, in Petri dishes, were inoculated by dusting with aeciospores from these anemones. Infection took place and uredinia containing urediniospores typical of *Tranzschelia punctata* were produced in 12 to 20 days on all of the hosts except cherry. The aecial stage of the stone-fruit rust has not been reported previously from the Pacific Coast, but specimens of cultivated anemones bearing aecia, collected at Salem, Oregon, in 1926, and received from H. P. Barss, as well as similar material collected near San Jose, California, in 1927, by L. R. Cody, are deposited in the pathological herbarium of the California Department of Agriculture.

Black scorch of the date palm.—L. J. KLOTZ and H. S. FAWCETT.

A fungus disease of economic importance has been found on date palms in California, Arizona, and Northern Africa. Preliminary survey indicates that all varieties of the date palm probably are susceptible. The disease has been found occurring naturally on all parts of the plant except the roots and stem, and these latter organs have, by artificial inoculation, been found readily susceptible. The most typical lesion is a dark brown to black, hard carbonaceous, scorching effect on petioles, midrib, and fruit strands and fruit stalks, which suggests *black scorch* as the common name. Many of the fruit strands may be completely severed by the attack and the crop materially lessened. Wounding was shown to be unnecessary for infection of fruit strands. The decay is most serious where it attacks the terminal bud, either killing the palm or, when not fatal, producing the so-called "fool-disease" effect, in which the injured terminal bud grows out laterally, setting the normal growth of the palm back several years. Both the hyaline and the brown spores of the fungus *Thielaviopsis* sp., probably *T. paradoxa* (De Seynes) von Höhnel, are found on the surface of the lesions. The conidia originate endogenously in uniseriate chains from subhyaline conidiophores. The optimum temperature for fungus in culture lies between 24° C. and 27½° C.; it makes very little growth at 32° C. The brown spores apparently need a rest period before germination. The hyaline conidia germinate readily without a rest period, sending out one, occasionally two, germ tubes from any place on its periphery. In germinating, the protoplast of the mature macroconidium bursts through a longitudinal slit liberating a globule of naked protoplasm which proceeds to grow into mycelium. The hyphae are subhyaline with cross walls and show a strong tendency to anastomose and form branches at right angles to the parent hyphae.

To control the malady, the affected fronds, leaf bases, and inflorescences should be pruned out and the pruning cuts and surrounding tissues disinfected. Preliminary experiments indicate that copper sprays and dusts may be effective.

Graft transmissions of curly top in tomatoes (tomato yellows).—M. SHAPOVALOV.

Young, healthy tomato plants, about 3–4 inches high, grown in pots under greenhouse conditions at Berkeley, were inoculated by exposing one leaf of each plant to

viruliferous beet leaf hoppers, *Eutettix tenellus* Baker, for 1 week. The inoculated plants were then grafted by the approach method with healthy plants of the same age. Some of these plants were shaded. On the average a higher number of transmissions was obtained by inoculation with viruliferous insects than by grafting. The percentage of graft-transmitted cases of the disease was nearly equal to that of the insect-transmitted cases when grafts were made immediately after the removal of the insects. When there was an interval of 2 or more days between these two operations, a certain percentage of the grafts failed to show symptoms of the disease. Some of the plants that did not show the symptoms also failed to show the presence of the virus when tested with nonviruliferous insects fed afterwards to healthy sugar beets. Exposure of inoculated and grafted plants to additional artificial light in the evening, produced by four 500-watt Mazda lamps, for the duration of 4 to 6 hours, accelerated the rate of disease development, whereas muslin shading retarded it. No significant changes appeared in the final percentages of the disease.

Streak, a virus disease of peas transmitted by Thrips tabaci.—M. B. LINFORD.

A disease of canners' peas, *Pisum sativum* L., characterized by a streaked and spotted brown necrosis of pods, stems, and leaves, was found by the writer, in 1928, widely distributed across the United States. On pods necrotic circular pitting may develop, or the whole pod may collapse. On leaves the injury may begin with spotting or with brown vein streaking extending down the stems as a phloem necrosis. The pods alone, the stem apex, or the entire plant may become necrotic. No microorganisms have been found associated with this disease. During investigations of pineapple yellow spot in Hawaii, infective thrips, *Thrips tabaci* Lindeman, were transferred to peas from infected *Emilia sagittata* (Vahl) DC. Symptoms developed that appear identical with pea streak and that do not follow feeding of noninfective thrips. Of 45 plants so treated, 21 have become infected. Individual thrips have transmitted infection. Thrips reared on infected peas have transmitted to peas, reproducing streak, and to pineapple, producing typical yellow spot. In peas the incubation period is about 12 to 20 days. Streak caused by the yellow-spot virus occurs in market-garden plantings near Honolulu. It is suggested that streak on the mainland is caused by either this or a related virus.

Further studies of transmission of the pineapple yellow-spot virus by Thrips tabaci.—M. B. LINFORD.

Further studies of the transmission of the pineapple yellow-spot virus by *Thrips tabaci* Lindeman have revealed a specialized relationship between virus and insect. *Emilia sagittata* (*E. flammea*) was the chief test plant, supplemented by pineapple and others. The thrips were from three noninfective colonies reared each from an individual larva. The yellow-spot virus is transmitted by both adults and large larvae reared on infected plants. The virus survives pupation. A single insect may transmit. When adults from noninfective colonies are allowed to feed upon diseased plants they appear never to become infective. Progeny of these adults, allowed to develop on the same diseased plants, do become infective. Also, larvae from noninfective colonies allowed to feed upon diseased plants become infective and may transmit the virus while either larvae or adults. The failure of adults to become infective has been shown consistently in tests with thrips from a uniform source exposed to four species of host plants as the source of virus. In the larvae there is an incubation period lasting approximately 10 days. This virus is not readily transmitted by mechanical means. Host plants of the yellow-spot virus include members of several unrelated families.